CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

W. F. GEDDES, Editor-in-Chief
R. J. TARLETON, Managing Editor } University Farm, St. Paul 1, Minn.
EUNICE R. BROWN, Editorial Asst.

Editorial Board: J. S. Andrews, W. B. Bradley, G. Garnatz, I. Hlynka, Majel M. MacMasters, H. S. Olcott, J. A. Shellenberger, Betty Sullivan

CONTENTS

PAGE

Preparation and Chemical Characteristics of the Cohesive Proteins of Wheat, Barley, Rye, and Oats. *D. K. Cunningham, W. F. Geddes, and J. A. Anderson* 91

Grain Storage Studies. XVIII. Mold Invasion of Wheat Stored for Sixteen Months at Moisture Contents below 15 Percent. *Clyde M. Christensen* 107

The Storage Stability of Vacuum-Packed Active Dry Yeast. *A. R. Felsher, R. B. Koch, and R. A. Larsen* 117

Bacterial Aspects of Soda Cracker Fermentation. *Jan Micka* 125

The Effect of Temperature on the Rate of Penetration of Moisture within Damped Wheat Grains. *J. D. Campbell and C. R. Jones* 132

The Inhibition of Wheat Lipoxidase by Cyanide. *G. N. Irvine and J. A. Anderson* 140

Viscosity vs. Protein and Ash Content of Western Wheat Varieties. *C. R. Bresson and M. A. Barmore* 144

Gas Pressure in Fermented Doughs, *C. H. Bailey* 152

A Note on Jojoba Bean Meal—A Potential Feed. *Franklin B. Wells* 157

Book Reviews 159

Editorial Policy and Suggestions to Authors 164

Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 16, 1934. Published at 500 So. 5th St., Minneapolis, Minn.

Vol. 32 March, 1955 No. 2

MIXING ATTACHMENTS

FOR YOUR Hobart

The cut to the right shows the Hobart #A120 equipped with our Model CT-W-120 Bowl and Fork including water jacket. All bowls of this series are made so that if purchased without water jacket same may be added at any time.

This mixing equipment provides, among other things, a clearance between mixing surfaces of 1/4". This gives more of a STRETCHING mixing action than the 1/16" to 1/8" clearance employed in the Model known as the "McDuffee Bowl and Fork" which we have made in recent years.

For HOBART A200 Model CT-W-200	Water Jacketed Bowl, Stainless Fork	$95.00
For HOBART A200 Model CT-200	Plain Bowl, Stainless Fork	$65.00
For HOBART A120 Model CT-W-120	Water Jacketed Bowl, Stainless Fork	$90.00
For HOBART A120 Model CT-120	Plain Bowl, Stainless Fork	$60.00
For HOBART C10 Model CT-W-10	Water Jacketed Bowl, Stainless Fork	$80.00
For HOBART C10 Model CT-10	Plain Bowl, Stainless Fork	$50.00

CEREAL CHEMISTS' EQUIPMENT MANUFACTURER

NATIONAL MFG. COMPANY

LINCOLN, NEBRASKA

Noted For Uniformity and Light Color

Your tests will confirm the quality of these Merck Vitamin Mixtures

In the mill or in the control laboratory, MERCK VITAMIN MIXTURES stand the most critical examination. They are light in color, uniform in feeding characteristics, and handle accurately in all types of equipment. Preferred for these advantages by hundreds of mills, MERCK VITAMIN MIXTURES are readily available from stocks strategically located at:

Atlanta, Ga.; Buffalo, N. Y.; Rahway, N. J.; St. Louis, Mo.; Dallas, Texas; Knoxville, Tenn.; Chicago, Ill.; Seattle, Wash.; Kansas City, Mo.; Los Angeles, Calif.; Oklahoma City, Okla.; Ogden, Utah; Denver, Colo.; Minneapolis, Minn.

Merck Vitamin Mixtures

FOR FLOUR ENRICHMENT

FIRST TO CONTAIN THIAMINE MONONITRATE

Research and Production for the Nation's Health

MERCK & CO., INC.
Manufacturing Chemists
RAHWAY, NEW JERSEY

© Merck & Co., Inc.

Champions are proven

that's why millers have confidence in

Whether it's a product or a blue ribbon setter, champions are proven *in the field!* And that's true—even in the science of producing phosphates. Take V-90, for example. Here's a real veteran with more than 16 years of actual field experience. No other phosphate equals its performance.

During the past 16 summers—some of them the most severe ever encountered—self-rising products have been tested in practically every section of the nation. And so has V-90! *Not once* has it failed to give the outstanding leavening performance expected of it.

All this didn't just happen. V-90 is the result of Victor's 57 years of leadership in the field of leavening. V-90 has passed every field and laboratory test for stability.

Yes, champions are *made*—not born. But, it takes *field tests* to *prove* a champion! That's why now, more than ever before, millers have confidence in V-90.

CHAMPION IN THE FIELD . . . FOR QUALITY AND STABILITY

THE PHOSPHATE CRYSTAL WITH A "HAIR BOAT"*

V-90®

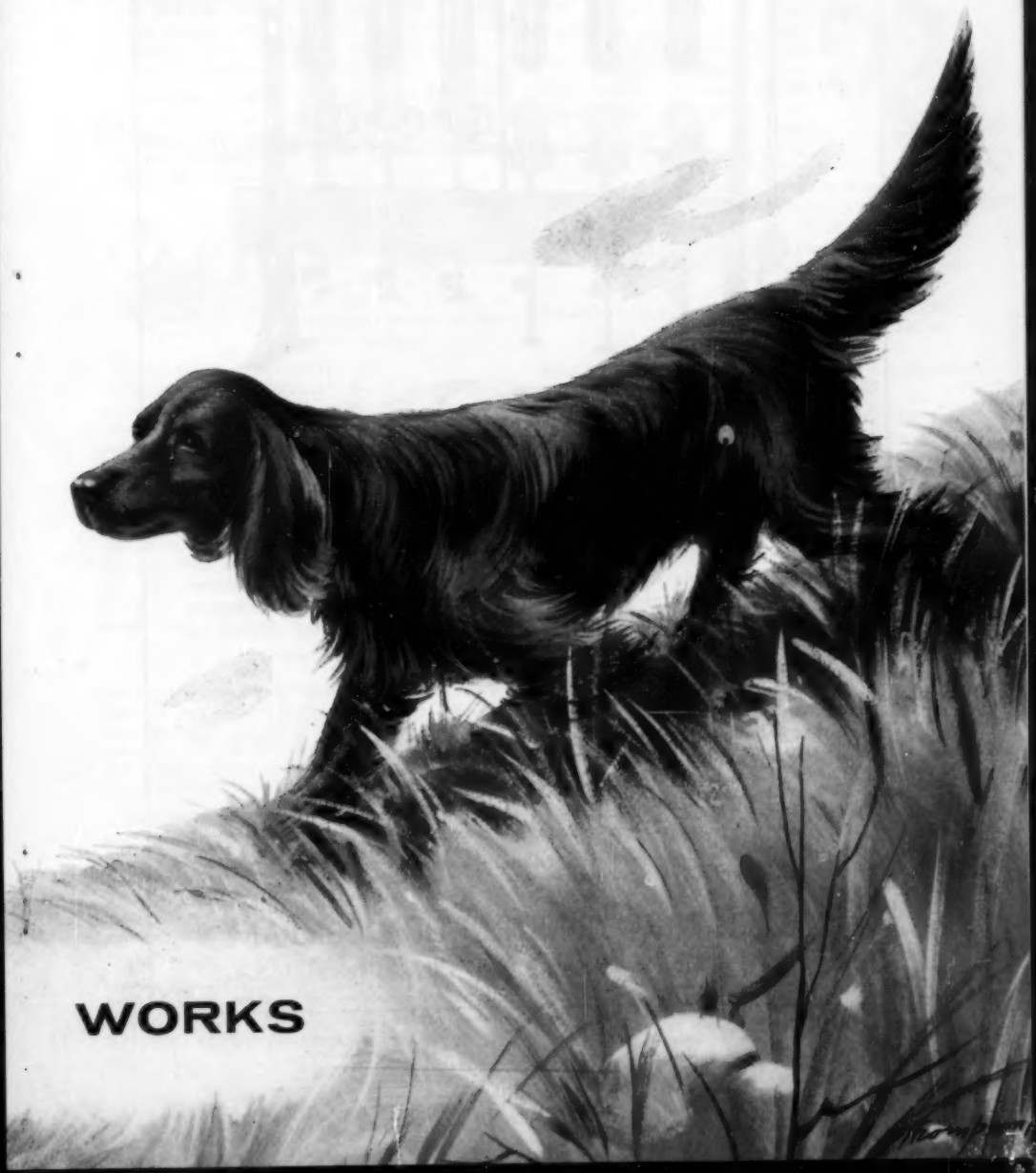
*Coated Anhydrous Monocalcium Phosphate

VICTOR CHEMICAL

155 N. WACKER DRIVE CHICAGO 6, ILLINOIS

in the field!

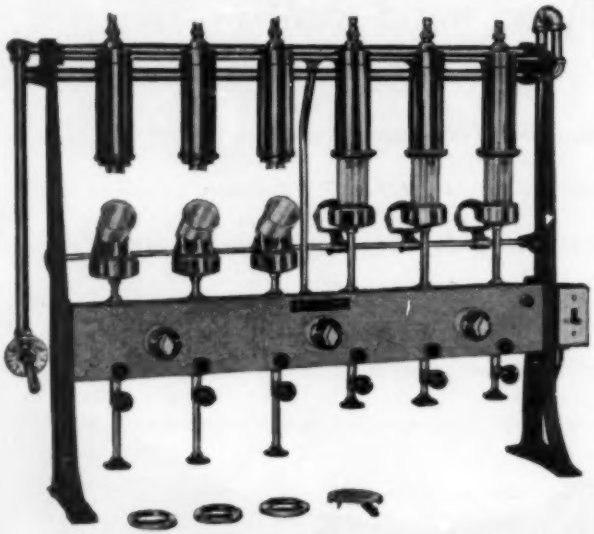
V-90



WORKS

Automatic Release and Seal
SPEEDS SOLVENT EXTRACTIONS

FOR FATS, VITAMINS, CAROTENE AND OTHER DETERMINATIONS



NO. 3000 "GOLDFISCH" EXTRACTION APPARATUS

Dimensions: 16" x 38" x 31" high. Available in 2, 4, and 6 capacity units, complete with all glassware and ready to operate.

In "Goldfisch" improved extraction apparatus, the condensation chamber is sealed automatically after air has been automatically released by the valve. This permits vaporized solvent to condense quickly in the condensation chamber and speeds your extraction process. The sealed chamber also prevents the escape of solvent so that a high percentage can be reclaimed.

These time-saving, money-saving features of "Goldfisch" extraction apparatus make it the choice of hundreds of industrial and institutional laboratories. "Goldfisch" apparatus is in service today in 30 state feed control laboratories.

Comparative tests, list of users, full details in Folio "F".
Write today for your copy.

Also available: Bulletin on Kjeldahl Nitrogen apparatus and crude fiber condensers.

LABORATORY CONSTRUCTION COMPANY

1113–1115 Holmes Street, Kansas City, Missouri, U.S.A.

The Vital Story of BREAKFAST CEREALS

with essential vitamins and minerals restored

by Science Writer

This article is one of a series devoted to the story of vitamin enriched or restored cereal products: white bread and rolls, white flour, corn meal and grits, macaroni products, white rice, breakfast cereals, farina.

Increasing in popularity over the years, breakfast cereals are used throughout the United States by young and old alike. Eaten with fruit and milk or light cream, they contribute an excellent combination of basic, flavorful, nutritious foods to the diet of children and adults.

In the past decade the use of these foods has grown markedly. In fact, it is estimated that there has been a 20% increase during the period. In 1953 the increase was much higher than the average of previous years. It was 5.5%.

During 1953, for example, many tons of breakfast cereal foods were eaten in the United States. Industry figures show that 4.6 pounds of dry cereals and 2.5 pounds of hot (cooked) cereals per person were consumed.

Many grains are processed to make breakfast cereals: wheat, corn, rice, oats. Often these foods may be eaten just as they come from the manufacturer's package. Or they may require only a short period of cooking to be ready for the table. They appeal to busy home-makers, as well as to institutional dietitians, because they are readily available in a variety of flavors to add interest and value to an important, but sometimes neglected, meal. They are used in cookery, too.

A Nutritional Advance

As the science of nutrition advanced in recent years, it was found that losses of essential vitamins and minerals occurred during processing of wheat, corn and rice into breakfast foods. When this became know, most manufacturers acted to overcome the losses. They adopted restoration.

Restoration simply means that certain vitamins and minerals are added to the cereal food during processing so that the finished product is generally equal to the whole grain values of those elements. The vitamins thiamine (B_1), riboflavin (B_2), and niacin as well as the mineral, iron, are used in restoration. Vitamin D is also added sometimes.

The recent trend toward pre-sweetened cold cereals for breakfast consumption and between-meal snacks emphasizes the nutritional importance of vitamin fortification. Increased calories require more "B" vitamins for proper utilization.

What the Vitamins Do

Thiamine is the vitamin which helps to build and maintain physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

Niacin, which is another of the "B" vitamins, is needed for healthy body tissues. Its use in the American diet has done much to make a serious disease called pellagra disappear.

Riboflavin is the vitamin which is essential for growth. It helps to keep body tissues healthy and to maintain proper function of the eyes.

Vitamin D is important to prevent rickets in children and the development of certain abnormal bone conditions in adults.

Iron is essential for making good red blood and for preventing nutritional anemia.

Where Do The Vitamins Come From?

At about the same time that processing losses in breakfast cereals became known, other developments in the science of nutrition produced ample supplies of vitamins at economical prices. Thus, the quality of breakfast cereal foods could be, and was, greatly improved through restoration.

Since the early days of breakfast food restoration and of white flour and white bread enrichment, the world-famous firm of Hoffmann-La Roche has supplied top quality vitamins by the tons. Pioneering work in its laboratories and by its collaborators resulted in the "duplication" of some of Nature's extremely complex substances. First, the chemical composition of the vitamin was learned. Second, the pure substance was isolated. Third, the "duplicate" was made by synthesis. And fourth, the laboratory techniques were extended to large scale operations.

The manufactured "duplicate" is identical chemically and in biological activity with Nature's own product. A vitamin is still a vitamin regardless of its source. So efficient is large scale manufacturing that vitamins are sold at a lower cost than if they were extracted from natural sources.

Reprints of this article, and of all others in this series, are available without charge. Please send your request to the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

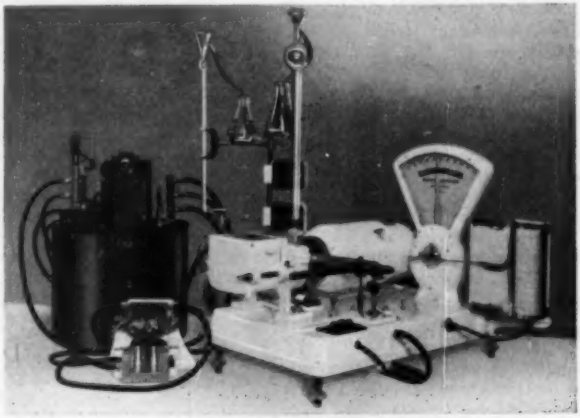
We frankly admit to this fault——

we are sticklers for perfection.

We have designed a cooling equipment for the cooling water of the Thermostat. The function of this cooling equipment is to make the operation of the FARINOGRAPH more automatic and relieve the operator of having to watch the temperature.

The cooling equipment is placed between the tap water and the Thermostat and insures perfect temperature control in the Thermostat — automatically — even on the hottest days.

The cooling equipment is now undergoing thorough testing and as soon as the "bugs" are ironed out, it can be had at a very nominal rental per month. It will also become standard equipment on all future FARINOGRAPHS.



You do not have to purchase a FARINOGRAPH to use one. It is available on small monthly rental payments and you have the privilege of stopping these rental payments at any time and returning the instrument. Under these easy conditions — without financial risk to you — you cannot really afford *not* to have a FARINOGRAPH at your disposal all the time. We invite your inquiry.

BRABENDER CORPORATION Rochelle Park, N.J.

You can order all these essential bakery products

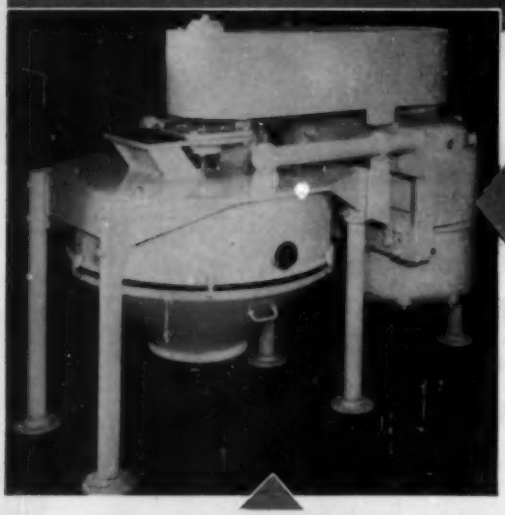
National Bakers Compressed Yeast
National Active Dry Yeast
National Baking Powder
National Baking Cream
National Bakers Margarine

National Bakers Malt Syrup
National Bakers Dry Malt
National Enrichment Tablets
National Yeast Food
National Puff Paste

National 7-in-1
Armour Cloverbloom Frozen Whole Eggs
Armour Cloverbloom Frozen Egg Whites
Armour Cloverbloom Armtex
Armour Cloverbloom Sugared Yolks

500 CWT PER HOUR!!!

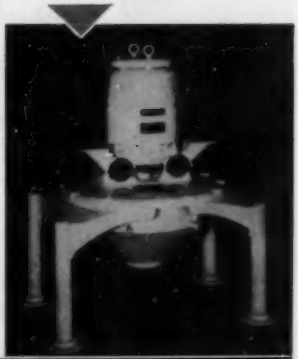
it's a cinch with the NEW "BIG INCH"....



for Positive Infestation Control . . .

the NEW "ENTOLETER" 'BIG INCH' for 150 to 500 cwt per hour

the STANDARD "ENTOLETER" unit for 25 to 150 CWT per hour



You guarantee your flour specifications... let "ENTOLETER" Infestation Control assure the complete cleanliness of all ingredients and finished products . . .

. . . the trademark "ENTOLETER" on milling equipment is your guarantee of complete satisfaction. Write to us for information concerning our products. ENTOLETER DIVISION, The Safety Car Heating and Lighting Company, Inc., 1153 Dixwell Ave., New Haven 4, Connecticut.

The trademark "ENTOLETER" is your guarantee of complete satisfaction

ENTOLETER
CENTRIFUGAL MACHINES

CEREAL CHEMISTRY

VOL. 32 MARCH, 1955 NO. 2

PREPARATION AND CHEMICAL CHARACTERISTICS OF THE COHESIVE PROTEINS OF WHEAT, BARLEY, RYE, AND OATS[1]

D. K. CUNNINGHAM, W. F. GEDDES, AND J. A. ANDERSON

ABSTRACT

Viscoelastic proteins have been prepared from wheat, barley, and rye flours by first extracting the flours with distilled water and then with dilute formic acid in a Waring Blendor. Neutralizing the formic acid extract with saturated calcium hydroxide solution precipitates the proteins which may be gathered up in a cohesive form when preparations are made from wheat, barley, and rye flours. Oat flour by this procedure yields only a claylike protein. Optimum yields with least damage were obtained with 0.01 *N* formic acid; oxalic and citric acids were no more efficient than formic acid. Effective extraction apparently requires strong shearing forces, as only low yields were obtained by gentle methods of extraction. Increasing amounts of sodium bisulfite increased and then decreased the solubility of wheat gluten in dilute formic acid, but caused a progressive increase in the solubility of oat protein.

Barley and rye "glutens" were firmer but less cohesive and extensible than analogous wheat glutens. Oat proteins were claylike rather than cohesive. Proteins of barley and rye darkened in color more rapidly than those of wheat and oats. The nitrogen contents of all four preparations were about 16%; reprecipitation did not alter their nitrogen contents significantly. In amide nitrogen content oat "gluten" was lowest, wheat gluten highest, and barley and rye were intermediate. The water-absorbing powers of wheat and rye proteins by formic acid extraction were 65.0 and 70.3% compared to 55.2 and 50.2% for barley and oat proteins.

One of the fundamental problems of dough technology is to find a chemical explanation of the viscoelastic and cohesive properties of wheat-flour doughs. Since the cohesiveness of wheat-flour dough is believed to reside mainly in the proteins, an attempt has been made to obtain further insight into the nature of dough by comparing the properties of wheat protein with those of analogous proteins from other cereals. By modification of Lusena's procedure (7), crude but analogous preparations of "gluten" were obtained in this laboratory from wheat, barley, rye, and oats (1). At about the same time Hagberg (3) reported on a similar preparation for rye only.

[1] Manuscript received September 28, 1954. Joint contribution from the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and the Department of Agricultural Biochemistry, Institute of Agriculture, University of Minnesota, St. Paul, Minnesota. Paper No. 137 of the Grain Research Laboratory, No. 318 of the Associate Committee on Grain Research (Canada), and No. 3231 of the Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota. Condensed from part of a thesis presented to the Faculty of Graduate Studies of the University of Minnesota by the Senior Author in partial fulfillment of the requirements for the Ph.D. degree, June, 1954.

The present paper deals with an extension of this investigation involving detailed studies of the acid extraction of the proteins from flours and an examination of certain properties of "glutens" precipitated from acid extracts by saturated calcium hydroxide.

Materials

Early experiments were made on casual samples of wheat, barley, rye, and oats available in the laboratory. They were milled on an Allis-Chalmers experimental mill to yield a flour of good dress practically free from bran particles. Yields and protein contents (N $\times$5.7, dry basis) for this *first series* of flours were: Thatcher wheat, 55 and 15.3%; Montcalm barley, 35 and 9.3%; rye (mixed varieties), 45 and 7.8%; and Ajax oats, 30 and 10.9%.

In collecting a *second* series, flours of similar protein content were obtained for all cereals except rye, for which a sample of high enough protein content could not be obtained. Protein contents of these flours were: wheat, 11.0%; barley, 11.1%; rye, 8.9%; and oats, 11.2%.

Extraction of Protein from Cereal Flours

General methods used for extraction are described in the next subsection. Studies of possible modifications of Lusena's method are then presented in subsections dealing with: acetic acid extraction; formic acid extraction; effects of prior extraction with water; a comparison of formic, oxalic, and citric acids as extracting agents; effects of mild agitation; and the effects of combining bisulfite with formic acid. In general, the comparisons were made by determining the total nitrogen in the various fractions and by setting up nitrogen balance sheets.

General Methods. Flour samples were slurried with the required amount of acid for 5 minutes at full speed in a Waring Blendor with a variable transformer for speed control. Wet gluten was dispersed for 2 minutes at low speeds, until lumps were broken up, and then at full speed for 3 minutes.

Preliminary water extractions, when required, were made in 250-ml. centrifuge tubes by slurrying with a small laboratory stirrer for the required time. After centrifuging, the residue was extracted in the Waring Blendor. The foam was broken by addition of 1 or 2 drops of *n*-octyl alcohol, and the mixture was centrifuged again. Floating scum was then removed by rapid filtering through a pad of fine glass wool. These filtered solutions gave a negative test for starch.

Precipitation with saturated calcium hydroxide was carried out with a Fisher Electrometric Titrimeter with which continuous pH measurement could be made during addition of the precipitating

agent. Further discussion of the precipitation with saturated calcium hydroxide will be found in the section dealing with precipitation.

After precipitation, the gluten mass was gathered up and adhering water was pressed out with the fingers. Notes were made on the properties and texture of the preparations as these were observed by visual and tactile methods during preparation.

Nitrogen distribution was determined on suitable aliquots, after excess water was evaporated, by the standard Kjeldahl method. For determination of total solids, other aliquots were evaporated to dryness in porcelain dishes; ash was determined on the residues by incineration of the dry solids in a muffle furnace.

Extraction with Acetic Acid. Lusena's observations were tested by extraction of the first series wheat flour with 0.005 *N* acetic acid. About 50% of the protein was extracted, and excellent gluten was recovered by neutralization with saturated calcium hydroxide solution. Negligible amounts of protein were extracted from barley, rye, and oat flours with 0.005 *N* acetic acid; even with 0.01 *N* acid, only 5 to 10% of the protein was extracted, and no protein could be recovered from the extracts by neutralization.

Lusena had indicated that water extraction of the flours prior to extraction with acid reduced the content of water-soluble ash and permitted greater recovery of the gluten. Twenty-five grams of flour were slurried with 200 ml. of water for 10 minutes. The dispersion was then centrifuged, and the solids redispersed in 0.01 *N* acetic acid with the Waring Blendor. For wheat, the dispersion of protein was increased to 80%, and the gluten recovered by precipitation approximated the yield of hand-washed gluten from the same flour, namely 85%.

When this pre-washing procedure was applied to barley, rye, and oat flours, extraction was increased, but little protein could be recovered on subsequent neutralization. No improvement was obtained by variation of the washing procedure.

Extraction with Formic Acid. Mangels (9) noted that the protein-solubilizing power of an organic acid was decreased by an increase in the chain length of the acid, which suggested that formic acid might be a more efficient protein solvent.

When washed flours were extracted with 0.01 *N* formic acid and neutralized, somewhat larger yields of protein were obtained from wheat, and much larger yields from barley, rye, and oats. Wheat flour yielded a white elastic gluten similar to gluten prepared by Lusena's acetic acid method. Barley and rye flours yielded substances resembling wheat gluten but less elastic and cohesive; but oat flour yielded

a claylike material which showed no tendency to become a gluten.

The distribution of nitrogen among the fractions thus obtained is presented in Table I. The data show that rye flour has a much larger water-soluble protein fraction than wheat, barley, or oats; whereas barley flour has a large fraction insoluble in water and formic acid.

TABLE I

NITROGEN DISTRIBUTION IN EXTRACTS OF CEREAL FLOURS

Nitrogen	Wheat, Thatcher	Barley, Montcalm	Rye, 1 C.W.	Oats, Ajax
	% of total	*% of total*	*% of total*	*% of total*
Soluble in water	11.1	12.5	35.7	13.1
Soluble in 0.01 *N* formic acid	85.2	56.2	51.0	63.9
Insoluble in 0.01 *N* formic acid	3.7	31.3	13.3	13.0
	% of flour	*% of flour*	*% of flour*	*% of flour*
Total — dry-matter basis	2.7	1.6	1.4	2.3

Prior Water Extraction. A more detailed study was next made of the effect of varying the degree of water extraction. Wheat flour, representing a gluten-forming cereal, and oat flour, which had so far given no glutenous protein, were extracted first with water for 5, 60, and 120 minutes and then with 0.01 *N* formic acid by the usual method. Analytical data for this experiment are given in Table II. The total solids extracted by water from wheat increased regularly when extrac-

TABLE II

EFFECT OF VARYING THE DURATION OF WATER EXTRACTION ON SOME FRACTIONS OF WHEAT AND OAT FLOURS

(All quantities expressed as percent of flour)

Extract	Wheat			Oats		
	5 min.	60 min.	120 min.	5 min.	60 min.	120 min.
	%	%	%	%	%	%
	Total Solids					
a. Water extract	4.1	4.9	5.1	3.9	4.5	4.4
b. Supernatant from protein precipitation	5.2	4.7	3.4	1.6	1.7	1.4
	Protein					
a. Water extract	1.3	1.4	1.6	1.2	1.5	1.4
b. Acid extract	7.2	9.0	9.0	3.6	3.9	3.8
c. Supernatant from protein precipitation	3.1	2.8	1.8	0.4	0.4	0.4
	Ash					
a. Water extract	0.2	0.2	0.3	0.3	0.3	0.3

tion time was extended to 2 hours, while the total solids extracted from oats increased up to 60 minutes, then decreased at 2 hours.

The protein extracted from wheat flour by water washing, the amount of protein subsequently extracted by formic acid, and especially the yield of gluten (as shown by reduced nitrogen in the supernatant liquid) were increased by extending washing time. By difference, the gluten yields in mg. per g. of flour are: for 5 minutes' washing, 41.9 mg.; for 60 minutes' washing, 62.2 mg.; and for 120 minutes' washing, 72.4 mg. Oats differs from wheat in that after 60 minutes no significant change in acid-extracted protein or in precipitated protein takes place.

The water-soluble ash, expressed as mg. per g. of flour, increased for wheat with extraction time, paralleling the increases in total solids, water-soluble protein, and acid-soluble protein. For oats, continued extraction after 60 minutes decreased the soluble ash, and there is a parallel though slight decrease in total solids, water-soluble protein, and acid-soluble protein. The percentage of total ash extracted by water varied from 38.4 to 52.7% for wheat and from 28.0 to 30.8% for oats. Much longer washing times, or several changes of water, did not increase the yield of acid-soluble protein for oats. This indicates that the remaining mineral matter in oat flour is firmly bound; and no method of removing it has yet been devised.

A water-extraction time of 1 hour was adopted for all further work.

Comparison of Formic, Oxalic, and Citric Acids. Since formic acid proved to be a protein dispersant, the analogous members of the di- and tri-carboxylic acid series also appeared to merit study. For the dicarboxylic acids, oxalic is the first member, and for tricarboxylic acids, citric acid was employed as a hydroxy derivative of the true primary member, tricarballylic acid. Tests were made on the second series of flour samples of 11% protein level. Wheat glutens prepared with either 0.005 *N*, 0.01 *N*, or 0.02 *N* formic acid appeared similar in quality to hand-washed gluten; those from oxalic acid were weaker and less extensible; and those from citric acid were more extensible but less elastic. The barley and rye glutens prepared with oxalic acid were tougher than the formic acid glutens, while those prepared with citric acid were more extensible.

Table III shows that maximum extraction was obtained with 0.02 *N* acids, except for wheat flour extracted with formic and oxalic acids, and for rye flour using citric acid, in which 0.01 *N* acids gave slightly higher extractions. Although 0.01 *N* oxalic acid was a better extractant for barley and rye flours than was 0.01 *N* formic acid, the insolubility of the calcium oxalate made the use of 0.01 *N* formic acid

preferable; 0.01 *N* formic acid was therefore selected for further studies.

TABLE III
COMPARATIVE DISPERSING POWER OF THREE ORGANIC ACIDS

Acid Used	Normality	Total Protein Extracted			
		Wheat	Barley	Rye	Oats
		%	%	%	%
Formic	0.005	75.6	51.8	49.6	24.8
	0.01	85.2	56.2	50.0	36.5
	0.02	84.3	62.6	54.5	67.9
Oxalic	0.005	85.7	23.9	52.2	3.5
	0.01	83.9	64.1	56.8	40.0
	0.02	82.0	66.2	57.1	50.0
Citric	0.005	69.8	15.2	52.2	10.5
	0.01	81.4	41.4	57.4	22.1
	0.02	83.6	58.7	54.6	64.5

TABLE IV
pH OF CEREAL PROTEINS DISPERSED IN 0.01 *N* FORMIC, OXALIC, AND CITRIC ACIDS

Acid Used	Cereal Protein			
	Wheat	Barley	Rye	Oats
Formic	3.2	3.3	3.2	3.3
Oxalic	2.8	2.9	3.0	2.7
Citric	3.9	4.0	4.2	3.8

Since the variation in protein dispersing power might be attributed to varying acidity, or ability to produce hydrogen-ions, of the three acids, Table IV shows the pH of dispersions of wheat, barley, rye, and oat proteins in formic, oxalic, and citric acids. The differences between formic and oxalic extracts are small, but citric extracts have a somewhat higher pH.

Effect of Mild Agitation on Extraction of Cereal Proteins. Dispersion of proteins in the Waring Blendor causes foaming, and it is possible that denaturation of the protein and loss of solubility might occur through exposure to air at the vastly increased surface. Effects of milder agitation were examined with a shaker rotating four 250-ml. centrifuge bottles at 15 r.p.m. Washed flour samples of 25 g. were shaken with 25 g. of glass beads, 6 mm. in diameter, and 200 ml. of 0.01 *N* formic acid for 2 hours. The bottles were centrifuged; an aliquot of the supernatant was analyzed for nitrogen, and the remainder of the protein was precipitated with saturated calcium hydroxide solution.

The amounts of total protein extracted were: wheat, 20%; barley, 5%; rye, 25%; and oats, 62%. Neutralization produced a noncohesive protein for oats and rye, and a gelatinous, slightly cohesive protein for wheat. No precipitate formed from the barley extractions. Extending the time of shaking to 24 hours did not increase the amount of oat or rye protein solubilized but increased the wheat extraction to 24% and the barley to 8% respectively. This method of extraction merited no further study.

Effect of Bisulfite on Extraction. Recent work by Hlynka (5) indicated that bisulfite in low concentrations disintegrates wheat gluten. Foster, Yang, and Yui (2) have shown that complete solubilization of corn proteins by means of detergents can be effected only if small amounts of bisulfite are added. A study was undertaken to determine whether small additions of bisulfite would promote more complete solubilization of wheat protein by formic acid, and since oat protein was least soluble, oat flour was also included in the experiments. The washed flour was treated in a Waring Blendor for 5 minutes with 400 ml. of 0.005 *N* formic acid containing varying amounts of sodium bisulfite. The foam was broken with 2 drops of *n*-octyl alcohol, and the liquid was centrifuged. Protein determinations were made on 50-ml. aliquots from the protein extraction after diluting to 500 ml. The results are shown in Table V.

TABLE V

EFFECT OF ADDED BISULFITE IN SOLUTION OF WHEAT AND OAT PROTEIN BY 0.005 *N* FORMIC ACID

Bisulfite	Total Protein Solubilized	
	Wheat Flour	Oat Flour
Mg.	%	%
0	85.2	24.8
2	92.2	30.2
5	88.9	37.3
10	85.9	38.2
15	84.5	40.2
20	...	41.9
40	...	43.8
50	...	57.6
80	...	59.9

The data indicate that wheat protein interacts with sodium bisulfite initially to give a substantial increase in solubilized protein. With succeeding increments of bisulfite the solubility decreased steadily. Oat protein, on the other hand, showed a substantial increase on

addition of bisulfite, and succeeding increments increased the solubility of the protein. While bisulfite acted as an effective adjunct to increase both wheat and oat protein solubility, it was of no further value to the work since it tends to disintegrate wheat gluten.

Properties of "Glutens" Precipitated by Calcium Hydroxide

The "glutens" precipitated from formic acid by calcium hydroxide solution were examined for color, consistency, and other subjective data; for total nitrogen content of glutens treated in various ways; for amide nitrogen; and for the water-absorbing capacity of some "glutens."

General Methods. Precipitation was carried out in all cases by addition of filtered saturated calcium hydroxide solution to the stirred acid solution of protein. During precipitation, the pH was observed by means of a continuous reading electrometric titrimeter with the electrodes remaining in the solution being titrated. When the desired pH was attained, usually from pH 5.5 to 6.0 depending on the cereal protein, the stirrer and electrodes were removed and the solution was centrifuged to recover the precipitated protein.

Wheat, barley, and rye proteins, collected into a mass in the centrifuge bottle by means of a rubber policeman, were placed in evaporating dishes and freeze-dried in a large vacuum desiccator. The proteins were then dried under vacuum over anhydrous calcium sulfate. Wheat glutens puffed up to form a fluffy structure which crumbled easily to a powder; barley and rye glutens puffed to some extent but dried to a hard substance; and oat protein dried to a powder. The wheat, barley, and rye proteins were ground to pass a 40-mesh sieve, whereas the oat protein required no further treatment.

Observed Properties of the Glutens. The most outstanding differences between proteins were the cohesiveness and elasticity of the wheat, barley, and rye "glutens," as compared to the claylike oat gluten. Precipitated wheat gluten resembled hand-washed gluten except that it was whiter and appeared to be slightly less elastic. Barley and rye glutens were very firm and inelastic, although they were sufficiently cohesive to be clearly differentiated from the oat protein. The wheat gluten darkened slowly to the light gray color characteristic of hand-washed gluten; but barley and rye glutens darkened rapidly to a dark brown shade. Oat protein darkened somewhat to a light brown color.

Nitrogen Content of the Glutens. It is difficult to wash out wheat gluten by hand to much above 90% protein, calculated on an $N \times 5.7$ basis, and it has therefore been assumed that a considerable amount of nonprotein material is bound in hand-washed gluten. Lusena found

the protein content of a typical lyophilized gluten to be 90% and thus showed that purified gluten, which contained negligible amounts of starch, might be no higher in nitrogen than hand-washed gluten. Corresponding data on similar "gluten" preparations from other cereals have not previously been available.

Data for the protein preparations made from water-extracted flours by a single extraction with formic acid and precipitation with calcium hydroxide solution are shown in Table VI for both series of flours

TABLE VI

NITROGEN AND PROTEIN CONTENTS OF PRECIPITATED CEREAL PROTEINS
(Dry-matter basis)

Cereal	Protein in Flour (N × 5.7)	Precipitated Cereal Proteins	
		Nitrogen	Protein (N × 5.7)
	%	%	%
First series			
Wheat	15.3	16.1	91.7
Barley	9.3	14.0	79.8
Rye	7.8	14.4	82.0
Oats	10.9	14.7	83.8
Second series			
Wheat	11.1	15.3	87.2
Barley	11.3	13.7	78.0
Rye	8.4	14.6	83.2
Oats	11.0	15.2	86.5

employed in these studies. Nitrogen determinations were made on protein samples dried *in vacuo* over anhydrous calcium sulfate and then stored over phosphorus pentoxide. Some variations occur in the protein content of these preparations. Tests showed that starch was not present and could not contribute to the variations.

Similar studies (Table VII) were made of "gluten" prepared with other acids. The citric acid preparations differed from the formic in containing slightly less nitrogen, on a percentage basis, while the oxalic acid preparations were definitely very much lower in nitrogen owing to the presence of occluded calcium oxalate. The oxalic acid preparations were therefore analyzed for calcium by ashing a sample of the protein, dissolving the ash, precipitating the calcium as calcium oxalate, and titrating with standard potassium permanganate solution. The calcium was calculated as calcium oxalate, and this was used to determine the corrected nitrogen content.

The correction for calcium oxalate in the oxalic acid preparations accounts in part for the low nitrogen content but does not bring the

TABLE VII

NITROGEN CONTENT OF PROTEIN PREPARED BY EXTRACTION WITH VARIOUS ACIDS
(Dry-matter basis)

Cereal	Formic	Citric	Oxalic	
			As Found	Corrected
	%	%	%	%
First series				
Wheat	15.3	15.1	13.0	13.7
Barley	14.9	14.6	12.2	13.4
Rye	14.6	14.4	9.2	11.7
Oats	14.7	14.5	13.4	14.0
Second series				
Wheat	15.2	14.9	11.7	...
Barley	14.7	14.6	12.1	...
Rye	14.6	14.4	9.9	...
Oats	15.2	14.9	12.7	...

nitrogen values up to those found for the other acids. Extraction with oxalic acid apparently allows more of the non-nitrogenous fraction to solubilize and precipitate with the protein or, alternately, oxalic acid may possibly solubilize a different fraction of gluten.

Nitrogen Content of Retreated Glutens. The hypothesis that the low nitrogen content of the cereal proteins might be caused by the presence of nonprotein contaminants was tested by triple solubilization and precipitation of the proteins prepared from the first series of samples. The data in Table VIII indicate that slight increases in total

TABLE VIII

EFFECT OF REPRECIPITATION ON THE NITROGEN CONTENT OF CEREAL PROTEIN PREPARATIONS
(Dry-matter basis)

Cereal Protein	No. of Precipitations	Nitrogen Content	Protein on N × 5.7 basis
		%	%
Wheat	1	15.5	88.5
	2	15.4	87.5
	3	15.1	86.0
Barley	1	14.8	84.2
	2	14.9	84.9
	3	15.1	86.0
Rye	1	14.5	82.8
	2	14.7	83.6
	3	14.6	83.1
Oats	1	14.7	84.0
	2	15.2	86.4
	3	15.4	87.6

nitrogen may be obtained by repeatedly redissolving and reprecipitating barley and oat proteins, but a slight decrease takes place in wheat proteins when so treated. It thus appears that the contaminant is bound to the protein.

Amide Nitrogen Content of Gluten. The amide nitrogen of proteins arises from the ammonia combined with the free carboxyl groups of glutamic and aspartic acids. The content of amide nitrogen, when compared with the content of these dicarboxylic acids, gives an indication of the number of free carboxylic groups and serves as a check on the values obtained by titration methods.

Amide nitrogen was determined by deamidation of the protein and subsequent removal and estimation of free ammonia. For deamidation the method of Rees (11) was used. Free ammonia was estimated by the procedure of Middlebrook (10). In the final method 0.2 g. of gluten were hydrolyzed in 20 ml. of concentrated hydrochloric acid at 37°C. for 220 hours. The hydrolysates were made up to 50 ml. and 5 ml. were taken for distillation into saturated boric acid solution and subsequent titration by the method of Ma and Zuazaga (8).

In order to determine whether the cereal proteins were similar in their rate of release of amide nitrogen, a comparison was made of

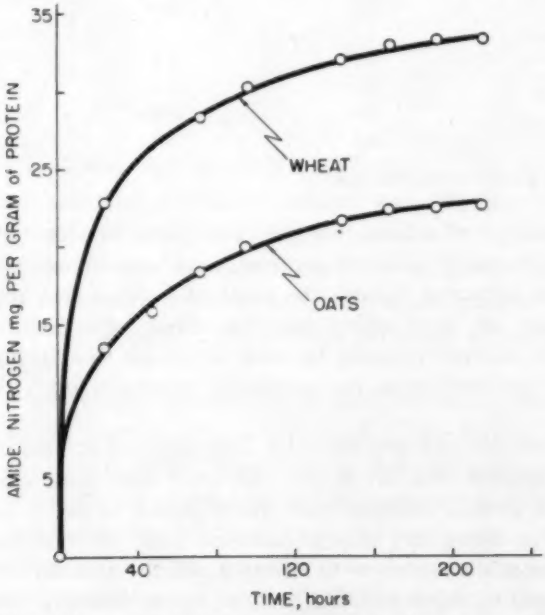


Fig. 1. Effect of time of hydrolysis on formation of amide nitrogen from wheat and oat proteins.

wheat and oat proteins prepared by extraction with formic acid. In this study, 1 g. of protein was treated with 100 ml. of concentrated hydrochloric acid and stored at 37°C.; 5-ml. aliquots were withdrawn at intervals for ammonia determinations. The rate of production of free ammonia is shown in Fig. 1.

The curves show that at least 160 hours are required to release almost all amide nitrogen from wheat and oat proteins and that from this time nitrogen appears to be released at a constant rate. On the basis of these curves an arbitrary digestion time of 200 hours was selected for future work.

Table IX gives data for a series of proteins from various flours.

TABLE IX

AMIDE NITROGEN OF PROTEIN PRECIPITATIONS

(Dry-matter basis)

Description of Gluten	Amide N	Total N	Amide N[a]
	%	%	%
First series			
Wheat	3.3	16.1	20.5
Barley	2.4	14.0	17.3
Rye	2.4	14.4	17.0
Oats	1.9	15.0	12.8
Second series			
Wheat	3.2	15.3	20.8
Barley	2.6	13.7	19.1
Rye	2.5	13.6	18.0
Oats	2.3	15.2	14.9

[a] Expressed as percent of total nitrogen.

The proportion of amide nitrogen is highest in wheat protein, intermediate in barley and rye proteins, and least in oat protein. This gradation is apparent in both the series of samples and appears to be characteristic of these cereal proteins. Here also, confirmation of Heathcote's analysis (4) may be seen, although this author analyzed whole oat protein with no particular emphasis on "true" amide nitrogen.

Water-Absorbing Characteristics. The dried wheat gluten absorbed a definite amount of water to give the usual semielastic material. Barley and rye glutens differed from wheat gluten in being tougher and firmer and in being very slow to reabsorb water when thoroughly dry. But oat gluten did not seem to absorb a definite amount of water.

In examining water-holding capacity some difficulty was found in determining when to weigh the wet "glutens." It was arbitrarily de-

cided to mold up the centrifuged gluten to remove surface moisture by rolling between dry fingers, and to weigh up a suitable portion. The moisture content was determined by the method of Hlynka and Anderson (6). For oat protein, the initial removal of excess water could not be readily effected. On prolonged centrifuging the oat protein became dry enough to be cut with a spatula, rather like cottage cheese. The moisture content of this dry material was over 70%, and water exuded from it on standing. It appeared that pressure might remove this moisture, and a moisture determination on the pressed material might more accurately represent the moisture content of the hydrated protein. Approximately 10 g. of oat protein were pressed between pads of filter paper at 50 lb. per sq. in. for 10 minutes; the moisture content was reduced to about 50% (Table X). Tests on wheat,

TABLE X

MOISTURE CONTENT OF THE GLUTENS

Cereal	Formic Acid Gluten		Oxalic Acid Gluten	
	Water in Wet Gluten	Water Absorption	Water in Wet Gluten	Water Absorption
	%	*g water/ g dry matter*	%	*g water/ g dry matter*
Rye	70.3	2.4	76.5	3.2
Wheat	65.0	1.8	65.2	1.9
Barley	55.2	1.2	59.1	1.5
Oats	50.2	1.0	48.3	0.9

barley, and rye glutens (first series of samples) indicated that pressing the gluten reduced the moisture contents only slightly: for wheat gluten, 2.2%; for barley gluten, 0.3%; and for rye gluten, 1.2%; whereas the reduction in moisture content of oat gluten was 28.6%. Table X shows the moisture content of unpressed glutens of wheat, barley, and rye and of pressed oat protein, prepared with both formic and oxalic acids.

Rye and wheat glutens possess higher moisture absorption capacity than barley or oat protein. This is true for both the formic acid series and for the oxalic acid series. The oxalic acid preparations are higher in moisture than the corresponding formic acid preparations for rye and barley, but the same for wheat; while the oxalic acid preparation from oats is slightly lower in moisture than the formic acid preparation. Some of these differences are probably the result of uncontrolled experimental error.

General Discussion

This work has shown that dilute formic, oxalic, and citric acids will solubilize most of the water-insoluble proteins of wheat, barley, rye, and oat flours. Of these acids, 0.01 *N* formic is the most efficient extractant of protein for wheat and oats; at this strength formic acid was only slightly inferior to oxalic acid for barley and rye protein. Acetic acid does not function efficiently under these conditions as a protein solvent except with wheat. Accordingly, it appears that either the molecular size or the configuration of the acid molecule contributes to their effectiveness; and in this respect the tendency of the carboxylic acids to form dimers may be a significant factor.

That energy is required to solubilize the proteins, within reasonable time limits, is shown by the studies with the rotating shaker; on this apparatus the mixing action is very gentle as the bottles are slowly turned end for end, but mixing is increased by the action of glass beads. This gentle agitation does not peptize the proteins of wheat, barley, or rye to any practical extent; apparently the powerful shearing forces generated in the Waring Blendor are required to break secondary valences and solubilize these proteins.

Preliminary water extraction of the flours is required to render the water-insoluble proteins liable to extraction with acids, but the changes induced by extraction are not entirely clear. Proteins of wheat flour absorb water and become swollen; the insoluble gums of wheat and barley also imbibe water; so that water extraction may expand the flour particles and may aid their dispersion in acid solution. In addition, the data in Table II indicate that the solubility of wheat gluten increases with increasing time of water extraction. This increase in solubility is paralleled by an increase in water-soluble solids and in water-soluble ash. Removal of some water-soluble component probably aids in increasing the protein solubility, and later studies on the precipitation of glutens from acid solution strongly suggest that the water-soluble mineral salts are involved (cf. Lusena, 7).

Bisulfite promotes the solution of oat protein in formic acid, and initially promotes and then decreases the solubility of wheat gluten in dilute formic acid. Bisulfite was expected to exert a disintegrating action on gluten and to promote gluten dispersion. This may have occurred, but the dispersion was masked by the precipitating effect of the sodium cation.

The observations on the physical characteristics of the cereal proteins indicated that a gluten series had been prepared. Subjective comparisons indicated that wheat gluten was highly elastic and co-

hesive, barley and rye glutens were intermediate, and oat gluten was slightly cohesive and entirely inelastic. A panel of three judges ranked barley gluten more cohesive and elastic than rye gluten.

In total nitrogen content, wheat gluten was highest and tended to be followed by oat protein, with barley and rye proteins giving lower values. However, the differences are not great, nor very consistent. Accordingly, it appears that variations in physical characteristics cannot be attributed to variation in total nitrogen.

The amide nitrogen content of the wheat gluten was highest, barley and rye glutens were intermediate, and oat gluten was lowest. The parallel between this postulated series and the series based on physical characteristics is of some interest in view of hypotheses of polymer cross-linkages occurring through the interaction of amide groups.

In water-absorbing capacity rye gluten was highest, wheat gluten was intermediate, and barley and oat glutens were lowest. This relationship is true for the formic acid series and the oxalic acid series. However, comparisons between the series do not reveal any systematic relation. The values for oat and possibly barley proteins are comparable to those of crystalline proteins, whereas the values for wheat and rye are more in the order of gels. Probably much of this variation may be attributed to the water-absorption of carbohydrate gums associated with the proteins, rather than to variations in the water absorption of the protein moiety.

It should be noted that this study was confined to two sets of samples. While these samples are believed to be representative, results obtained with them cannot be considered adequate for generalization about differences between the cereals. For example, while substantial differences were found in the amide nitrogen contents of the wheat and oat samples examined, a far larger investigation, probably involving a representative series of varieties grown at several stations, would be required to establish that oats is consistently lower in amide nitrogen than wheat.

The investigations described in this paper deal in some detail with the extraction of cereal proteins with dilute acids and with the properties of the "glutens" precipitated by neutralization with calcium hydroxide. Since the properties of the glutens may be expected to depend on the method of precipitation as well as on the method of extraction, a detailed study of precipitation appeared to be a logical next step. This work has been completed and is being prepared for early publication.

Literature Cited

1. CUNNINGHAM, D. K., and ANDERSON, J. A. Preparation of "gluten" from barley and rye. Cereal Chem. **27:** 344 (1950).
2. FOSTER, J. F., YANG, J. T., and YUI, N. H. Extraction and electrophoretic analysis of the proteins of corn. Cereal Chem. **27:** 477–487 (1950).
3. HAGBERG, SVEN. Preparation of gluten from rye. Bull. Ecole Meunerie Belge **14** (1): 1–16 (1952).
4. HEATHCOTE, J. G. The protein quality of oats. Brit. J. Nutrition **4:** 145–154 (1950).
5. HLYNKA, I. Effect of bisulfite, acetaldehyde, and similar reagents on the physical properties of dough and gluten. Cereal Chem. **26:** 307–316 (1949).
6. HLYNKA, K., and ANDERSON, J. A. A machine for measuring the extensibility and resistance to extension of gluten. Cereal Chem. **23:** 115–134 (1946).
7. LUSENA, C. V. Preparation of dried native wheat gluten. Cereal Chem. **27:** 167–178 (1950).
8. MA, T. S., and ZUAZAGA, G. Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Ind. Eng. Chem., Anal. Ed. **14:** 280–282 (1942).
9. MANGELS, C. E., and MARTIN, J. J., Jr. Peptization of wheat flour proteins by organic acids. Cereal Chem. **12:** 149–157 (1935).
10. MIDDLEBROOK, W. R. The irreversible combination of formaldehyde with proteins. Biochem. J. **44:** 17–23 (1949).
11. REES, M. W. The estimation of threonine and serine in proteins. Biochem. J. **40:** 632–640 (1946).

GRAIN STORAGE STUDIES. XVIII.

MOLD INVASION OF WHEAT STORED FOR SIXTEEN MONTHS AT MOISTURE CONTENTS BELOW 15 PERCENT[1]

CLYDE M. CHRISTENSEN[2]

ABSTRACT

Aspergillus restrictus, a slow-growing member of the *A. glaucus* group, invaded seeds of various wheats stored in the laboratory at moisture contents of 13.5–15.0% for 16 months. The same fungus was isolated from a large percentage of seeds of some samples of wheat taken from commercial bins. It appeared to develop more in the germ than in other parts of the seed, and increased invasion of the seed by this fungus was associated with decrease in germination and development of brown color characteristic of "sick" wheat. This fungus was prevalent in some commercially stored samples in which damaged or "sick" wheat had developed. The presence of *A. restrictus* was relatively difficult to detect in seeds because the fungus grew poorly or not at all on the common agar media used to culture molds, but it developed fairly well when seeds in which it was present were cultured on malt agar containing from 15 to 20% sodium chloride. Since this fungus slowly invades seed having moisture contents of 13.5% and above, the present generally accepted "critical" moisture levels for long-time storage of wheat (14.5% for hard red spring wheats, 14.0% for other wheats) appear to be too high.

Bailey and Gurjar (2) showed that respiration of spring wheats increased rapidly at moisture contents above about 14.5%. Coleman and Fellows (4) found that wheat in equilibrium with a relative humidity of 75% had a moisture content of 14.5–15.0%. Milner, Christensen, and Geddes (6) stated: "Moisture contents in wheat below about 14.5% (corresponding to a relative humidity of 74–75%) yielded low and constant respiratory rates over extended time intervals, indicative of purely seed respiration." They found no increase in molds in seeds stored at moisture contents below 14.5%. None of these tests involved storage times of more than a few weeks.

Snow (7), however, found that several subspecies of the *Aspergillus glaucus* group grew on feeding stuffs stored at relative humidities between 65 and 70%. One of these, *A. echinulatus*, was found only in materials stored at R.H. 65%, while *A. repens* and *A. ruber* (subspecies of *A. glaucus* common in stored grain) grew in materials at 67

[1] Manuscript received September 13, 1954. Paper No. 3219, Scientific Journal Series, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

[2] Professor of Plant Pathology, Department of Plant Pathology and Agricultural Botany, University of Minnesota, Institute of Agriculture, St. Paul 1, Minnesota. The work here reported was supported in part by grants from Cargill, Inc., and from the National Millers Federation and Grain Trades Council. Numerous samples from commercial bins were generously furnished by Cargill, Inc.

and 70% R. H. Snow, Crichton, and Wright (9) found that a number of factors, such as relative humidity, length of the storage period, the balance and type of nutrients in various feeding stuffs, temperature, and type of molds present all influenced the minimum relative humidity at which molds would invade feeding stuffs. They observed mold mycelium developing on locust beans stored at R. H. 65% for 2 years. Snow (8) found that spores of *A. echinulatus* would germinate at R. H. 62–66% within one or two years; the temperature at which these tests were made was not given. Hummel *et al.* (5), working with mold-free and mold-inoculated wheat, showed that molds were mostly responsible for biochemical changes associated with deterioration of wheat stored at moisture contents of 14.9% and above. If moisture contents in commercial grain bulks commonly vary as much as reported by Christensen and Drescher (3), and if molds were found to grow in wheat stored at moisture contents of 14.5% and below, as some of the above evidence suggests, this might help explain cases of deterioration in wheat stored at presumably safe moisture contents.

So far as is known, no laboratory tests have been made on the microbiology of wheat stored for relatively long periods of time at moisture contents at and just below those considered "critical," and for this reason the work here reported was undertaken.

Materials and Methods

Wheat Samples. The hard red spring wheat was from a parcel of the variety Marquis grown for seed in the dry-farming region of Montana, with a high germination and low infection by storage molds. The samples of hard red winter, soft red winter, and white wheats were from commercial parcels that had been in terminal storage for some months at Omaha, Nebraska, Chicago, Illinois, and Albany, New York, respectively, prior to the beginning of the tests. Additional samples from commercial storage are described in the text. All of the samples from commercial storage had a lower germination percentage, a higher percentage of seeds infected with storage molds, and a higher mold count per g. of seed, than the hard red spring wheat.

Moisture Contents. These were determined by the two-stage air-oven method, as specified in *Cereal Laboratory Methods* (1). All moisture contents are given on a wet-weight basis.

Storage of Samples. Samples of hard red spring, hard red winter, soft red winter, and white wheats were adjusted to moisture contents of 13.5, 14.0, 14.5, and 15.0% and stored in 4-oz. medicine bottles with screw caps equipped with waxed-paper or rubber liners. In most cases the bottles were only three-fourths full.

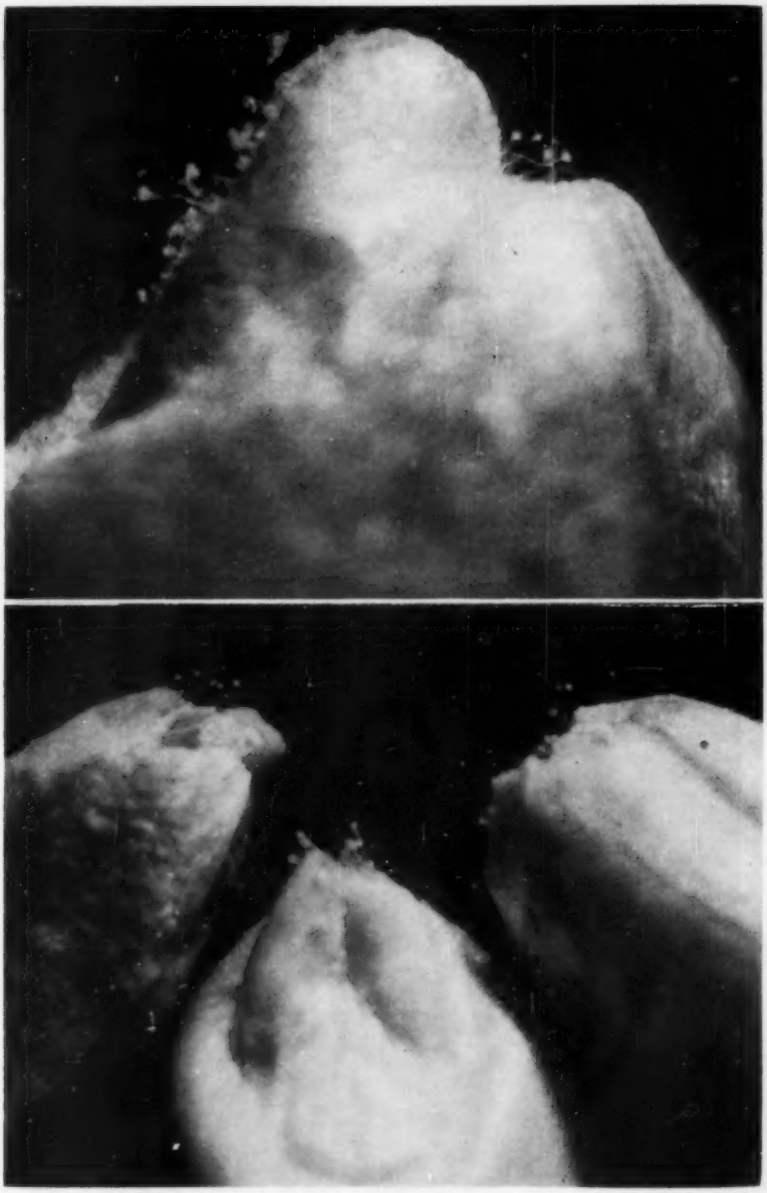


Fig. 1. Sporophores of **Aspergillus restrictus** growing from seeds of hard red spring wheat (upper), and hard red winter wheat (lower), stored at a moisture content of 13.5-14.0% for 16 months.

Germination. One hundred seeds were placed on moist paper and kept in closed enamel dishes at room temperature; the percentage of germination was determined after 6 days.

Number and Kinds of Molds Present. During the course of the tests the seeds were examined occasionally through the glass walls of the bottles, using a stereoscopic microscope with a magnification of $\times 10$ and $\times 20$. At the conclusion of the tests, 100 seeds were removed and examined in detail in the same way.

In culture tests, 100 seeds of each sample were cultured on malt agar containing 7.5% sodium chloride, and on malt agar containing 15–20% sodium chloride. Prior to culturing, the seeds were either (a) given no treatment; (b) washed thoroughly in running water plus detergent to remove most external contaminants; or (c) surface-disinfected by rinsing for 25–30 seconds in a 0.5% solution of sodium hypochlorite and rinsed in two changes of sterile water.

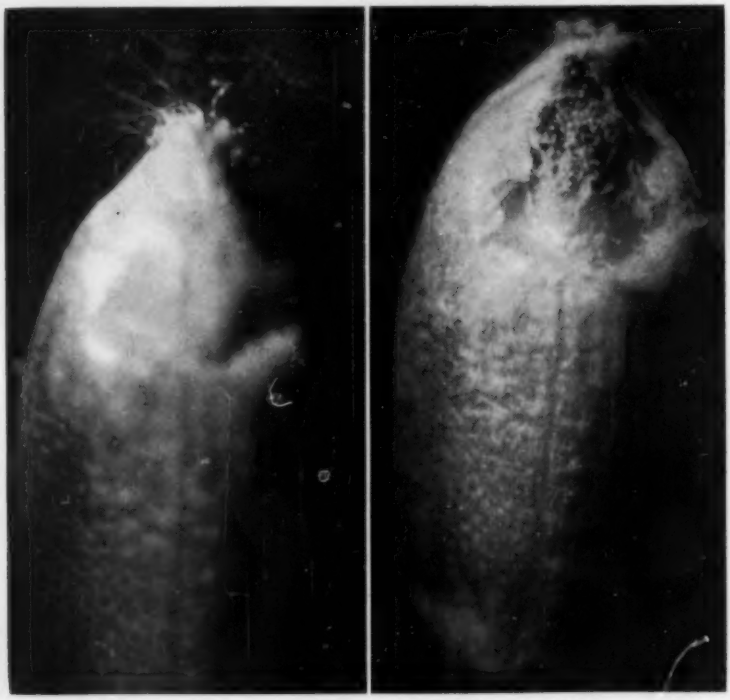


Fig. 2. *Aspergillus restrictus* growing from the germ of hard red spring wheat. Seed originally nearly free of storage molds. Seed stored at a moisture content of 14.0-14.5% for 16 months, surface-disinfected, cultured on malt agar containing 7.5% salt, and incubated 2 weeks. Left, sparse growth of mycelium externally visible. Right, the same seed with the pericarp removed, showing extensive development of mold on the germ.

Fig. 3. **Aspergillus restrictus** on hard red spring wheat. Upper, original seed stored dry in the laboratory. Lower, seed stored 16 months at a moisture content of 13.5-14.0%. Seed cultured without treatment on malt agar containing 18% sodium chloride. Photographed after 7 days' incubation.

Discoloration of Germ. This was determined by removing the pericarp above the germ from 100 seeds of each sample and examining the exposed germ with a stereoscopic microscope, using a magnification of ×10 and ×20.

Results

Samples Stored in the Laboratory. Within 16 months, sporophores of Aspergillus developed abundantly in the germs of all seeds stored at moisture contents of 14.5–15.0%, sparsely on most seeds at moisture contents of 14.0–14.5%, and on 11 to 78% of those at moisture contents of 13.5–14.0%. Typical seeds of both hard red spring wheat and hard red winter wheat stored at moisture contents of 13.5–14.0% for 16 months at room temperature are shown in Fig. 1. In Fig. 2 are shown seeds of hard red spring wheat that had been stored for 16 months at a moisture content of 14.0–14.5%, surface-disinfected, cultured on malt agar containing 7.5% sodium chloride, and incubated for 2 weeks at room temperature. Even after this long incubation period almost no mycelium had grown out onto the agar, although sparse mycelium and a few sporophores developed from the germ region. When the outer pericarp was removed from these seeds, *Aspergillus restrictus* was found to have grown extensively in and fruited heavily on the germs.

When cultured on malt agar containing 15% sodium chloride, many of the seeds stored for 16 months at moisture contents of 13.5–14.5% yielded *Aspergillus restrictus.* In Fig. 3 are shown seeds of the original hard red spring wheat, and those of the sample stored at 13.5–14.0% moisture, both cultured without pretreatment on malt agar containing 15% sodium chloride. Significant data from these tests are summarized in Table I.

In most cases, "normal" strains or subspecies of *Aspergillus glaucus,* mainly *A. repens* and *A. ruber,* invaded 100% of the seeds stored at moisture contents of 14.5–15.0%; in those seeds stored at moisture contents of 13.5–14.0% these subspecies of *A. glaucus* decreased from a moderately high proportion in the original seeds to a very small amount. The original seed of hard red spring wheat contained very little *A. glaucus,* and thus could exhibit no significant decrease in the amount of this fungus present.

Samples from Commercial Bins. Approximately 40 samples of soft red winter and white wheats, taken by probe, were obtained from commercial bins in Chicago, Illinois, Maumee, Ohio, and Albany, New York. When taken from the bins the moisture contents of these samples ranged from 13.5 to 14.7%. These were stored for 2 to 3

TABLE I

PERCENTAGE OF GERMINATION, MOLD INVASION, AND GERM DISCOLORATION OF WHEATS STORED 16 MONTHS AT ROOM TEMPERATURE AND AT MOISTURE CONTENTS OF 13.0–15.0 PERCENT

Wheat	Moisture Content		Germination	Seeds with Sporophores on Germ	Seeds Yielding Aspergillus restrictus when Cultured	Seeds with Discolored Germs
	Original	Final				
	%	%	%	%	%	%
Hard red spring	10[a]		96	0	0	0
	13.5	12.4	91	0	0	0
	14.0	13.2	89	22	100	0[d]
	14.5	14.0	17	82	100	16
	15.0	14.5	0	100	100[b]	...[e]
Hard red winter	11[a]		89	0	0	0
	13.5	13.1	26	0	30	2
	14.0	13.6	7	76	100	12
	14.5	14.2	0	100	100	46
	15.0	14.4	0	100	100[c]	96
Soft red winter	11[a]		70	0	0	1
	13.5	13.1	42	0	0	3
	14.0	13.6	6	11	6	16
	14.5	14.1	0	78	80	55
	15.0	14.4	0	100	100	100
White	11.5[a]		60	0	10	0
	13.5	13.5	26	13	90	25
	14.0	14.1	5	94	100	57
	14.5	14.8	0	100	100	100
	15.0	15.4	0	100	100[c]	100

[a] Original seed.
[b] All A. repens.
[c] Mostly A. repens.
[d] 25% with base of germ discolored.
[e] Not determined.

months in the laboratory at room temperature, in the cans in which they had been placed when removed from the bins. Sporophores of *A. restrictus* developed on the germs of a high percentage of the seeds of some of these samples. Typical ones are shown in Fig. 4. When such

Fig. 4. **Aspergillus restrictus** growing from the germs of white wheat from a bin at Albany, N. Y. The wheat when taken from the bin had a moisture content of 14.4%, and contained 15% damaged or "sick" seed. Stored in the original moisture can for 2 months before photographed.

seeds were surface-disinfected and cultured on malt agar containing 15% sodium chloride and incubated for 2 to 3 weeks, the same fungus grew from a large percentage of them. *Aspergillus restrictus* was also isolated from a large percentage of seeds of certain probe samples immediately after they were taken from the bins, seeds whose moisture contents at that time ranged from 14.0 to 15.0%. It was especially prevalent in those samples whose germination was low and in which 10–30% damaged or "sick" wheat was present.

Discussion

Aspergillus restrictus invaded the seed of different wheats stored at moisture contents of 13.5–14.5% for 16 months. Such invasion appeared to be predominantly in the germs of the seed. In general, increasing invasion of the seed by this fungus was associated with decreasing germination and an increasing percentage of discolored germs similar to those encountered in "sick" wheat. The fungus was also isolated from a high percentage of some samples of wheat from com-

mercial bins, especially where some "sick" wheat was present, and it thus is not merely a laboratory phenomenon. Whether it is a direct cause of, or major contributing factor to, the development of "sick" wheat as it is encountered in the trade cannot be stated with certainty. The fact that an increasing percentage of brown germs accompanied increasing invasion of laboratory-stored samples by *A. restrictus* indicates that a causal relationship may exist. Additional tests designed to evaluate more accurately the role of this fungus in the deterioration of commercially stored wheat are now in progress.

Aspergillus restrictus was relatively difficult to detect by cultural means, even when it could be seen fruiting on the seed. Of more than a dozen agar media tested at various times during the work, including some (such as Czapek's agar containing 20% sucrose) used in mycological work to detect so-called lyophilic or halophilic molds, the best was malt agar containing 15–20% sodium chloride. In most cases the fungus, when inoculated onto agar media such as malt or potato-dextrose agar, failed to grow; it apparently prefers or requires an environment of high osmotic pressure. Thom and Raper (10, p. 137), in speaking of the *A. restrictus* series, state: "All of these forms, like many of the *A. glaucus* forms, grow characteristically under conditions of physiological drought—we have found them in many situations in which physiological drought is attained by physical dryness or osmotic concentrations attained by the presence of high percentages of sugar or sodium chloride." In attempting to determine the role of this fungus in the deterioration of grain or grain products, in the laboratory or under practical conditions, it would be desirable to devise a technic which would disclose its presence with a high degree of certainty.

The heretofore accepted "critical" moisture level for wheat apparently is the level at which the more rapidly growing subspecies of *Aspergillus glaucus,* such as *A. repens* and *A. ruber,* begin to grow and sporulate vigorously. In the present tests, the "critical" moisture level for the growth of *A. restrictus* in the seeds was approximately 13.5%. This is what would be expected from work recently reported (11) on the same fungus in barley seed and from other work cited, in which molds closely related to *A. restrictus* developed in various materials stored at R. H. 65–70%. Much wheat is stored commercially at an "average" moisture content close to 13.0%, and it is not unusual for portions of a given bulk to have a moisture content of 13.5% or more. The fact that *A. restrictus* was isolated from a large percentage of seeds from some commercial bins, and that some of these samples were

deteriorating, suggests that a moisture content in excess of 13.5% may not be safe for long-time storage of wheat.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Bailey, C. H., and Gurjar, A. M. Respiration of stored wheat. J. Agr. Research **12:** 685–713 (1918).
3. Christensen, C. M., and Drescher, R. F. Grain Storage Studies. XIV. Changes in moisture content, germination percentage, and moldiness of wheat samples stored in different portions of bulk wheat in commercial bins. Cereal Chem. **31:** 206–216 (1954).
4. Coleman, D. A., and Fellows, H. C. Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. Cereal Chem. **2:** 275–287 (1925).
5. Hummel, B. C. W., Cuendet, L. S., Christensen, C. M., and Geddes, W. F. Grain Storage Studies. XIII. Comparative changes in respiration, viability, and chemical composition of mold-free and mold-contaminated wheat upon storage. Cereal Chem. **31:** 143–150 (1954).
6. Milner, M., Christensen, C. M., and Geddes, W. F. Grain Storage Studies. VI. Wheat respiration in relation to moisture content, mold growth, chemical deterioration, and heating. Cereal Chem. **24:** 182–199 (1947).
7. Snow, D. Mould deterioration of feeding stuffs in relation to humidity of storage. Part III. The isolation of mould species from feeding stuffs stored at different humidities. Ann. Appl. Biol. **32:** 40–44 (1945).
8. Snow, D. The germination of mould spores at controlled humidities. Ann. Appl. Biol. **36:** 1–13 (1949).
9. Snow, D., Crichton, M. H. G., and Wright, N. C. Mould deterioration of feeding-stuffs in relation to humidity of storage. Part I. The growth of moulds at low humidities. Ann. Appl. Biol. **31:** 102–110 (1944).
10. Thom, C., and Raper, K. B. A manual of the Aspergilli, 373 pp. The Williams and Wilkins Co.: Baltimore, Md. (1945).
11. Tuite, J. F., and Christensen, C. M. Grain Storage Studies. XVI. Influence of storage conditions upon the fungus flora of barley seed. Cereal Chem. **32:** 1–11 (1955).

THE STORAGE STABILITY OF VACUUM-PACKED ACTIVE DRY YEAST[1]

A. R. Felsher, R. B. Koch, and R. A. Larsen

ABSTRACT

Granular and pelleted active dry yeast packed in vacuum were stored at —20, 0, 40, 70, 90, and 120 degrees F. (respectively, —29, —17.7, 4.4, 21.0, 32.1, and 49 degrees C.), and a weekly cycle of —20°F. (—29.0°C.), and 40°F. (4.4°C.).

As the temperature of storage decreased, the shelf life of the active dry yeast improved. Freezing temperatures had no effect on the baking quality of active dry yeast nor did alternate periods of freezing and thawing temperatures of —20°F. (—29.0°C.) and 40°F. (4.4°C.). When active dry yeast is stored at 40°F. (4.4°C.) or lower it is usable for bread baking after 2 years' storage. Yeast stored at 70°F. (21.0°C.) was usable for 21 months; at 90°F. (32.1°C.) the yeast was usable for 6 months; and at 120°F. (49.0°C.) the life was 8 days.

No significant difference was found between the storage stability of active dry yeast in pellet form and of that in granular form.

Experience has shown the desirability of feeding our soldiers fresh baked bread in the field. This objective is, however, beset with many difficulties, not least of which is the problem of supplying yeast to bakers in the field.

During World War II, this situation was greatly eased by the introduction of active dry yeast into the military supply system. Unlike moist compressed yeast, active dry yeast need not be refrigerated and is usable for baking after relatively long periods of storage.

In spite of these great improvements, there have been times when active dry yeast has not leavened a bread dough to the satisfaction of the military baker. This has raised a question as to the length of time active dry yeast can be stored and still be usable. The question has been a vexing one, which could not be satisfactorily answered by information in the literature (4, 5, 6, 7, 8). These studies were helpful but did not extend over long enough periods of time or wide enough ranges of temperature, and gave no information on the effect of repeated freezing and thawing on the stability of active dry yeast.

The problem of the military was broached to the six yeast companies producing active dry yeast. It was recommended that a storage test be set up for periods of time extending to 2 years, that the effect of low temperatures be particularly studied, and that the stability

[1] Manuscript received September 13, 1954. This paper reports research undertaken by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned number 474 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense.

characteristics of the two forms of active dry yeast – granular and pelleted – be investigated. In accordance with these recommendations, the present study was undertaken.

Materials and Methods

Three samples of a pellet yeast with a round hard surface and three of a granular yeast having an irregular shape and friable nature (Fig. 1) were supplied by the yeast producers. The samples were of the

Fig. 1. Left, pellet-type active dry yeast; right, granular-type active dry yeast.

best commercial quality obtainable and met the requirements of the military standard for yeast,[2] that is, a moisture content no higher than 8%, packing in an inert atmosphere, and good baking quality. Bak-

TABLE I

BAKING RESULTS SHOWING PRE-STORAGE QUALITY OF THE SIX YEAST SAMPLES USED IN THIS STUDY

Sample No.	Type of Yeast	Loaf Volume
		cc.
1	Pellets	2600
2	Pellets	2600
3	Pellets	2700
4	Granular	2750
5	Granular	2700
6	Granular	2525

[2] Military Specification, MIL-Y-10032a.

ing results obtained with these samples are shown in Table I. Storage trials were conducted on blends of the three samples of each type. The respective composites were thoroughly mixed, divided into 4-oz. portions, and sealed into 300 × 200 cans under vacuum (27 in. of mercury). In all cases, moisture content of the yeast was between 7 and 8%.

Cans of both types of active dry yeast were stored at —20°, 0°, 40°, 70°, 90°, and 120°F. (respectively —29°, —17.7°, 4.4°, 21°, 32.1°, and 49°C.), and a cycle of 1 week at —20°F. (—29°C.) and 1 week at +40°F. (4.4°C.). Bread baking tests were made on the stored samples at intervals of time up to 2 years; a fresh can was opened for each bread-baking experiment.

Activity of the yeast was measured by a baking test, employing the straight dough procedure. Formula and method were as follows:

Formula:

Ingredients	*Rehydration*	*Dough*
Active dry yeast	3.7 g.	
Water	50.0 ml.	190 ml. (variable)
Sugar	1.5 g.	18.3 g.
Salt	..	7.3 g.
Nonfat dry milk solids	..	7.3 g.
Shortening	..	10.9 g.
Flour[2]	..	365.0 g.

Rehydration. Active dry yeast was added to the sugar-water solution at 105°–110°F. (40°–43°C.); the mixture was allowed to stand at that temperature for 5 minutes, then stirred and allowed to stand for 10 minutes more at that temperature; stirred until the yeast was dispersed, and then added to the other combined ingredients.

Dough Mixing. The rehydrated yeast was added last to avoid the possibility of chilling the yeast. The dough was mixed until properly developed. The temperature of the dough after mixing was not less than 84°F. (28.8°C.) nor more than 86°F. (29.9°C.).

Fermentation and Baking. The dough was transferred to greased glass jars (calibrated in cc.) and allowed to rise in a cabinet maintained at 86°F. (29.9°C.) with humidity adjusted so as to prevent crusting of the dough. When the outer edge of a dough made with a standard active dry yeast reached the 1400-cc. mark, the time was noted. The dough was then punched and rounded. All of the test doughs, after they had fermented for the same length of time as the standard, were

[2] The flour used was a patent hard winter wheat analyzing 0.40% ash and 11.5% protein on a 14% moisture basis. The flour had a maltose value of not less than 275 nor more than 375 mg. maltose per 10 g. of flour. Analyses were made in accordance with procedures outlined in Cereal Laboratory Methods (1).

given identical treatments. The doughs were allowed to rest for 15 minutes at 86°F. (29.9°C.), then molded and placed in greased pans.[4] Proofing was done at 98°–102°F. (36.6°–38.8°C.) until the standard active dry yeast dough was 3/8 in. above the top of the pan. The required time was noted and test doughs were proofed for this length of time. Loaves were baked at 425°F. (218°C.) for 28 minutes and allowed to cool for 1 hour; loaf volume in cc. was determined by rape seed displacement. On the following day the bread was examined for grain, texture, and crumb color in comparison with the loaf made from the standard active dry yeast.

The sole criterion for measuring the loss of activity was loaf volume. As might be expected, when volume decreased, there was a corresponding change in the over-all appearance of the baked loaf. In the smaller loaves the crumb had a densely packed grain and this compactness caused changes in the texture and crumb color.

In the opinion of the authors, breads having loaf volumes smaller than 2200 cc. were unsatisfactory for eating purposes. While this volume was considered border-line, storage tests on active dry yeast samples giving lower volume were carried beyond this point in order to establish the reliability of the trend.

The standard error of the bread-baking test results reported in this work is ± 50-cc. loaf volume.

Results

Effect of Storage at Temperatures above 70°F. (21°C.). A rather rapid loss in activity in the active dry yeast stored at 120°F. (49°C.) is shown in Table II. After 8 days' storage at this temperature both

TABLE II

EFFECT OF STORAGE UNDER VACUUM AT 120°F. (49.0°C.) ON THE ACTIVITY OF GRANULAR AND PELLETED YEAST

Storage Time	Loaf Volume	
	Granular Yeast	Pelleted Yeast
Days	*cc.*	*cc.*
0	2525	2550
3	2700[a]	2625[a]
5	2450	....[b]
8	2200	2200
12	1700	2100
15	<1700	<1700

[a] Steam used in oven.
[b] Leaking can.

[4] Pan dimensions are as follows: top inside, 9⅞ by 4¼ in.; bottom outside, 9 by 3½ in.; depth, 2¾ in.

the granular and the pelleted yeast were producing bread of minimum quality, and after 12 days' storage the breads were completely unacceptable. This is a further demonstration of the fact already known that storage of active dry yeast at temperatures higher than 70°F. (21°C.) has a profound effect on yeast action (4, 6).

When the storage temperature was dropped to 90°F. (32.1°C.), the activity of the active dry yeasts stored in vacuum increased greatly. At

TABLE III

EFFECT OF STORAGE UNDER VACUUM AT 90°F. (32.1°C.) ON THE ACTIVITY OF GRANULAR AND PELLETED YEAST

Storage Time	Loaf Volume	
	Granular Yeast	Pelleted Yeast
Months	*cc.*	*cc.*
0	2525	2550
1	2375	2375
2	2250	2325
3	2300	2275
6	2300	....[a]
9	2100	2075
12	1875	1900

[a] Leaking can.

the end of the 6-month baking period, the yeast was still satisfactory for baking bread; beyond that time, loaf volumes fell below the acceptable range. At 12 months, the appearance of bread baked from these yeasts was very unsatisfactory (Table III).

The trend toward much greater stability at the lower temperatures

TABLE IV

EFFECT OF STORAGE UNDER VACUUM AT 70°F. (21°C.) ON THE ACTIVITY OF GRANULAR AND PELLETED YEAST

Storage Time	Loaf Volume	
	Granular Yeast	Pelleted Yeast
Months	*cc.*	*cc.*
0	2525	2550
3	2600	2575
6	....[a]	2425
9	2350	2450
12	2450	2575
15	2275	2250
18	2250	2225
21	2275	2275
24	1800	2050

[a] Leaking can.

continued as the temperature of storage was dropped further. Baking tests of yeasts stored at 70°F. (21°C.) only began to show signs of failure after 15 months of storage (Table IV). However, using the criterion of 2200-cc. volume, these yeasts were still satisfactory after 21 months of storage at 70°F. (21°C.). It was not until the yeast was stored for 2 years at 70°F. that a loaf of bread was produced which was considered unusable.

Effect of Storage below 70°F. (21°C.). Both granular and pelleted yeast were stored under vacuum at subfreezing temperatures – minus 20°F. and 0°F. (—29°C. and —17.7°C.) – and at the temperature of most refrigerators, 40°F. (4.4°C.). At all of these temperatures, the yeast remained usable over the 24-month storage period (Table V).

TABLE V

EFFECT OF STORAGE UNDER VACUUM AT LOW TEMPERATURES ON THE ACTIVITY OF GRANULAR AND PELLETED YEAST

Storage Time	Loaf Volume					
	−20°F. (−29.0°C.)		0°F. (−17.7°C.)		40°F. (4.4°C.)	
	Granular	Pelleted	Granular	Pelleted	Granular	Pelleted
Months	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
0	2525	2550	2525	2550	2525	2550
12	2650	2600	2500	2600	2600	2600
18	2475	2475	2475	2500	2500	2425
24	2375	2375	2400	2375	2350	2300

There appears to be a slight advantage in storing active dry yeast at sub-freezing temperatures.

Effect of Repeated Freezing and Thawing on Active Dry Yeast. Although a single freezing and thawing of active dry yeast did not affect

TABLE VI

EFFECT OF STORAGE UNDER VACUUM AT WEEKLY CYCLES OF —20° AND 40°F. (—29.0° AND 4.4°C.) ON THE ACTIVITY OF GRANULAR OR PELLETED YEAST

Storage Time	Loaf Volume	
	Granular Yeast	Pelleted Yeast
Months	*cc.*	*cc.*
0	2525	2550
1	2450	2350
2	2325	2450
4	2400	2700
6	2375	2525
9	2325	2400
12	2675	2500
15	2450	2400

its viability, it seemed possible that repeated freezing and thawing might be much more severe. Field reports from Arctic regions tended to support this assumption. This possibility was tested, and the results, reported in Table VI, did not support the assumptions. The temperature at which active dry yeast was stored could be cycled on a weekly basis, one week at —20°F. (—29°C.) and one week at 40°F. (4.4°C.), without any apparent ill effects.

Discussion

The sizes and external appearance of loaves baked with these active dry yeasts are illustrated in Fig. 2. Loaves 1, 4, 5, and 6 were judged

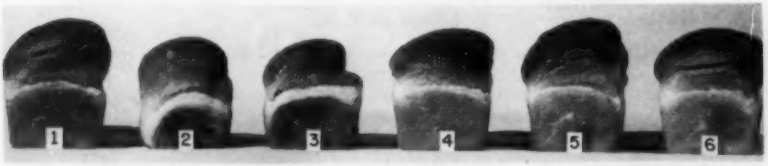


Fig. 2. Bread baked from active dry yeast stored under 27-in. vacuum for various lengths of time at several different temperatures: 1. Control, fresh standard sample, 2550 cc. 2. 10 days at 120°F., 1900 cc. 3. 8 months at 90°F., 2050 cc. 4. 21 months at 70°F., 2300 cc. 5. 21 months at 40°F., 2450 cc. 6. 21 months at 0°F., 2500 cc.

acceptable for eating purposes by the Armed Forces; breads 2 and 3 were not. The latter loaves had volumes of 1900 cc. and 2050 cc. respectively; these low volumes were related to the severe temperatures at which the active dry yeast was stored.

Undoubtedly, loss of leavening action of active dry yeast is related to destruction of living yeast cells. The minimum number of yeast cells that must be alive for adequate baking activity, as determined by plate count, is about 70%.[5]

Likewise, it is known that freezing of compressed yeast seriously impairs its use for baking (3). This knowledge and the fact that lyophilization of yeast kills 99.98% of the yeast cells (2) led the present authors to assume that active dry yeast could not be stored at freezing temperatures for long periods of time. When reports from the field suggested that loss in baking value of active dry yeast was associated with a series of freezings and thawings, the suggestion seemed plausible. That this research did not support this assumption came as a surprise.

These studies pose a very interesting question. Why is it that when yeast is dried in an unfrozen state and then frozen, the majority

[5] Personal communications (1954) from J. J. Enright, Mellon Institute, Pittsburgh, Pa.

of the yeast cells remain alive, whereas when yeast is frozen and then dried, most of the yeast cells are killed? This suggests an interesting field for research.

Acknowledgment

The authors are indebted to the active dry yeast task group of the Research and Development Associates, Food and Container Institute, Chicago, Illinois, whose cooperation made this study possible and who check-baked all the results reported in this study. They are Mr. Sebastian Lutz, Chairman of the Associates Task Group on Active Dry Yeast from the National Yeast Corporation, Belleville, New Jersey; Dr. Frank Rudert, Red Star Yeast and Products Company, Milwaukee, Wisconsin; Mr. Howard Clark, Standard Brands Inc., New York, N.Y.; Mr. Frank Feild, Federal Yeast Corporation, Baltimore, Maryland; Mr. Harold A. Meyer, Consumers Yeast Company, San Francisco, California; and Dr. Robert Summer, Anheuser-Busch, Inc., St. Louis, Missouri.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Atkin, L., Moses, W., and Gray, P. P. The preservation of yeast cultures by lyophilization. Wallerstein Labs. Commun. **12:** 365–367 (1949).
3. Bailey, L. H., Bartram, M. T., and Rowe, S. C. Effect of storage temperatures upon the viability and baking properties of compressed yeast. Cereal Chem. **17:** 55–66 (1940).
4. Crane, J. C., Steele, H. K., and Redfern, S. Techniques for estimating the stability of food products: Active dry yeast. Food Technol. **6:** 220–224 (1952).
5. Morse, R. E., and Fellers, C. R. Storage studies on active dried bakers' yeast. Food Technol. **3:** 234–236 (1949).
6. Oyaas, J., Johnson, M. J., Peterson, M. H., and Irvin, R. Effect of oxygen on retention of activity by commercial dried baker's yeast. Ind. & Eng. Chem. **40:** 280–283 (1948).
7. Quartermaster Board. Investigation of temperature and humidity conditions in long time storage facilities. Technical report, Quartermaster Board Series No. 206 (August 15, 1950).
8. Thiessen, E. J. The effect of temperature upon the viability and baking properties of dry and moist yeast stored for varied periods. Cereal Chem. **19:** 773–782 (1942).

BACTERIAL ASPECTS OF SODA CRACKER FERMENTATION[1]

JAN MICKA[2]

ABSTRACT

In soda cracker fermentation there is growth of the yeast which is added to the sponge, as well as bacterial growth derived from the flour and the dough adhering to the trough. Even in such low quantities as used in soda crackers (0.2–0.5%), yeast is a governing factor in fermentation. It acts as a starter, assisting the bacteria in the initial stage of sponge fermentation. However, after yeast growth reaches its optimum in 10–15 hours, both bacterial and yeast growth are retarded. Bacterial growth is retarded to a greater extent, so that yeast growth preponderates in the latter stage of sponge fermentation. These results suggest that there is a friendly association between bacteria and yeast and that soda cracker fermentation is a symbiosis rather than an instance of pure culture, at least in the early stages of sponge fermentation.

Bacterial as well as yeast growth in soda cracker fermentation affects pH and temperature rise in the sponge. It also affects pH and flavor of the finished product. Acidity formed during fermentation is developed largely by bacteria. It is favored by low percentage of yeast and retarded by high percentage of yeast. Sterile troughs retard bacterial and yeast fermentation and development of acidity. Percentages of yeast in excess of 0.50% and sterile troughs retard development of acidity in the sponge to a point resulting in a finished cracker with an abnormally high pH and an undesirable flavor.

There is relatively little in common in the production of bread and soda crackers aside from the fact that basically they are yeast-leavened. Whereas some bread is still "kitchen made" in homes and "hand made" in retail bakeries, soda crackers are produced only on a large commercial scale. Many attempts have been made to employ a higher percentage of yeast and a shorter sponge time in the manufacture of soda crackers, similar to that in commercial breadmaking, but without success; the finished product was different, especially in flavor, from that made by the traditional procedure. This difference may be due to bacterial fermentation, which probably plays a vital role in long-time sponges (18–24 hours) used in making soda crackers. In this respect soda-cracker fermentation may be similar to that of sour rye-bread doughs, where overnight sponges are used and bacterial fermentation is of utmost importance.

In such doughs Beccard (2) isolated a large number of species of lactic acid bacteria. Kent-Jones and Amos (3) studied the effect of bacteria and molds in wheat flour on panary fermentation. They found that even in long sponge processes, only one species of lactic acid bacteria, a strongly acid-forming *Streptococcus lactis,* may possi-

[1] Received for publication December 29, 1954.
[2] Research Department, United Biscuit Company of America, Melrose Park, Illinois.

bly be desirable even if it is not necessary. The desirable effect of lactic acid bacteria on soda cracker fermentation was reported by Micka (4).

In the present work, the relative growth of yeast and bacteria along with their effects on fermentation, pH, temperature increase of the soda cracker sponge, and the quality of the finished product was studied.

Materials and Methods

Preparation of Sample and Methods. The tests were carried out on commercial-size batches using 1000 lb. flour with 0.0, 0.125, 0.250, 0.500, and 0.750% baker's compressed yeast. The samples of sponge, for bacterial and yeast counts, were taken at the time the sponge was set (0 hour) and after 5, 10, 15, and 20 (at remix) hours of fermentation. Samples of dough were taken at the time the dough was mixed (set) and at the machine after 5 hours' proofiing time. Ten grams of dough were weighed into 90 ml. of water and transferred to a Waring Blendor and mixed for 4 minutes. Plating and counting were done according to the standard methods for dairy products (1).

Media. Difco nutrient agar of the following composition was used in making bacterial counts: 5 g. peptone, 3 g. beef extract, 15 g. agar, 1000 ml. water; pH, 6.8. Incubation was carried out at 37°C. for 48 hours.

Difco potato-dextrose agar was used for making yeast counts. Its composition follows: 15 g. agar, 20 g. dextrose, 200 g. infusion of potato, 1000 ml. water; pH, 3.5. Incubation was for 5 days at 20°C.

TABLE I

CHANGES IN BACTERIAL AND YEAST COUNTS, pH, AND TEMPERATURE IN CRACKER SPONGES AND DOUGHS MADE WITHOUT YEAST

	Time	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
	Hours					°C.
Sponge	0	13,400	11,100	400	6.0	22
	5	15,600	...	700	6.0	22
	10	210,500	...	2,400	5.8	23
	15	2,100,000	...	6,200	5.2	25
	20	17,640,000	16,300,000	10,800	4.6	27
Dough	0	14,610,000	12,800,000	8,100	6.5	28
	5	15,750,000	13,500,000	9,300	6.3	28
Cracker pH					6.6	

Results and Discussion

Bacteria and Yeast. In sponges set without yeast, the bacterial activity, though sluggish in the sponge stage, appears to favor the growth of acid-producers and the development of acidity (Table I). This results in a cracker with a low pH.

When 0.125% yeast (1¼ lb. per 1000 lb. flour) is used, both bacteria and yeast are relatively inactive during the first 10 hours of fermentation (Table II). Thereafter, activity of both is increased up to 15 hours

TABLE II

CHANGES IN BACTERIAL AND YEAST COUNTS, pH, AND TEMPERATURE IN CRACKER SPONGES AND DOUGHS MADE WITH 0.125 PERCENT OF COMPRESSED BAKER'S YEAST (FLOUR BASIS)

	Time	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
	Hours					°C.
Sponge	0	24,500	15,700	2,500,000	6.0	22
	5	25,000	...	3,100,000	5.9	22
	10	1,400,000	...	3,950,000	5.6	24
	15	24,600,000	...	15,700,000	5.3	27
	20	76,300,000	12,500,000	40,500,000	4.7	29
Dough	0	33,100,000	10,800,000	19,700,000	6.7	29
	5	21,500,000	8,700,000	14,300,000	6.5	29
Cracker pH					6.8	

and by 20 hours bacterial activity is predominating. Additional experience has shown that the fermentation is too vigorous and thus hard to control from sponge to sponge. The result is lack of uniformity in quality of the finished cracker. However, the accelerated activity is

TABLE III

CHANGES IN BACTERIAL AND YEAST COUNTS, pH, AND TEMPERATURE IN CRACKER SPONGES AND DOUGHS MADE WITH 0.25 PERCENT OF COMPRESSED BAKER'S YEAST (FLOUR BASIS)

	Time	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
	Hours					°C.
Sponge	0	22,500	18,000	5,850,000	6.0	22
	5	26,100	...	6,200,000	5.8	23
	10	6,200,000	...	13,400,000	5.3	25
	15	39,500,000	...	48,900,000	5.1	27
	20	30,700,000	11,200,000	46,500,000	4.8	28
Dough	0	28,100,000	10,000,000	42,700,000	6.7	28
	5	27,300,000	9,300,000	40,000,000	6.6	29
Cracker pH					7.0	

not carried over into the dough stage, where bacteria and yeast become sluggish if they function at all.

At the 0.25% yeast level (2½ lb. per 1000 lb. flour), the period of bacterial inactivity is shortened to 5 hours (Table III). Optimum activity is reached in 15 hours and in 20 hours there is a considerable decrease (22.3%) in bacterial activity and a slight decrease (4.9%) in yeast activity. Again, for all practical purposes, bacterial and yeast activity in the dough stage is nil.

Sponges set with 0.50% yeast (5 lb. per 1000 lb. flour) have a still shorter period of bacterial and yeast inactivity and optimum activity is reached in 10 hours (Table IV). After this time, yeast and bacterial

TABLE IV

CHANGES IN BACTERIAL AND YEAST COUNTS, pH, AND TEMPERATURE IN CRACKER SPONGES AND DOUGHS MADE WITH 0.50 PERCENT OF COMPRESSED BAKER'S YEAST (FLOUR BASIS)

	Time	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
	Hours					°C.
Sponge	0	24,000	18,500	12,200,000	6.0	22
	5	3,800,000	...	16,400,000	5.6	24
	10	46,500,000	...	65,700,000	5.2	27
	15	40,800,000	...	61,300,000	5.2	29
	20	37,200,000	8,500,000	54,200,000	4.9	29
Dough	0	32,500,000	6,800,000	51,100,000	6.8	29
	5	30,000,000	5,900,000	50,200,000	6.7	30
Cracker pH					7.5	

counts decrease. As in the previous instances, both forms of fermentation are virtually at a standstill in the dough stage.

In sponges set with 0.75% yeast (7½ lb. per 1000 lb. flour), bacterial and yeast activity begin almost immediately after mixing and increase rapidly up to 20 hours (Table V). Despite this progressive increase in over-all bacterial count, an indicated significant reduction in the acid-producers is apparent.

The data (Tables I–V) indicate that bacterial growth is governed by the amount of initial yeast inoculum. In the early sponge stage, the yeast acts as a starter, assisting the bacteria. The relative growth of bacteria in the first 10 hours of fermentation increases rapidly and almost geometrically with the percentage of yeast initially applied. Beyond the initial concentration of 0.5% yeast, it appears that the rate of growth is sharply retarded, apparently owing to exhaustion of the nutrient supply.

TABLE V

CHANGES IN BACTERIAL AND YEAST COUNTS, pH, AND TEMPERATURE IN CRACKER SPONGES AND DOUGHS MADE WITH 0.75 PERCENT OF COMPRESSED BAKER'S YEAST (FLOUR BASIS)

	Time	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
	Hours					°C.
Sponge	0	25,500	18,000	17,900,000	6.0	22
	5	8,300,000	...	25,400,000	5.5	24
	10	57,500,000	...	68,700,000	5.2	27
	15	62,700,000	...	83,600,000	5.2	29
	20	64,200,000	4,200,000	86,400,000	5.2	29
Dough	0	46,500,000	3,500,000	59,500,000	6.9	29
	5	41,800,000	2,300,000	54,300,000	6.8	29
Cracker pH					8.9	

Acid-producing bacteria, on the other hand, are retarded in their growth as the proportion of yeast is increased, especially in the later part of the sponge stage. The correlation coefficient between the end-pH of the sponges and the number of acid-producers at the end of sponge fermentation is $r = -0.982$, indicating that the acid-producers are mainly responsible for the acidity developed in the sponge.

pH and Temperature. Since the addition of yeast in setting the sponge increases bacterial growth during sponge fermentation, it seems reasonable to expect that it would enhance the decrease in hydrogen-ion concentration and the increase in temperature which occurs. Again, this effect is most pronounced in the first 10 hours of the fermentation period (Tables I–V). Both hydrogen-ion concentration and temperature increase as the percentage of yeast is raised, in a similar manner to that noted for the relation between bacterial growth and yeast percentage.

Acid-producers are strongly related to the acidity formed in sponge fermentation. Because of the retarding influence of the yeast inoculum on growth of acid-producing bacteria in the sponge, the end-pH of the sponge increases with percentage of yeast used initially. This effect carries over through the dough stage and, with still greater effect, into the finished cracker. In every case the change in pH, when related to percentage of yeast inoculum, is quite significant as is shown by the regression curves in Fig. 1. The mathematical expression of the regression curves is as follows: Percentage of initially applied proportion of yeast is represented by p; pH with the subscripts S, D, and C indicates end-pH of sponge, dough, and cracker respectively. Regression equations between these variables are:

	Standard Error of Estimate
S: $(pH)_S = 4.595 + 0.752\ p$	± 0.049
D: $(pH)_D = 6.383 + 0.607\ p$	± 0.069
C: $(pH)_C = 6.675 - 0.044\ p + 3.927\ p^2$	± 0.142

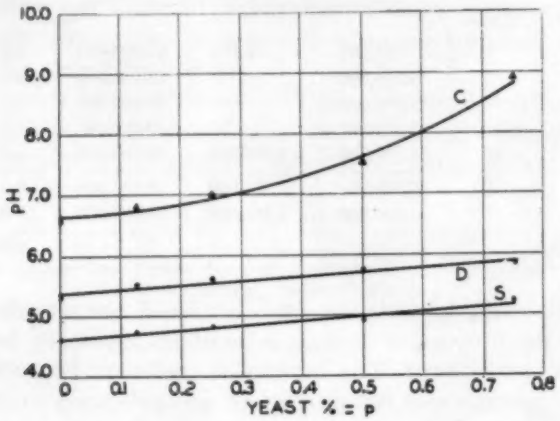


Fig. 1. Relation between the end-pH of sponges (S), doughs (D), and crackers (C) and the percentage of yeast employed (p).

TABLE VI

COMPARISON OF BACTERIAL AND YEAST COUNTS AND CHANGES IN pH AND TEMPERATURE OF SPONGES AND DOUGHS SET IN NORMAL AND STERILE TROUGHS

	Total Bacteria	Acid-Producers	Yeast	pH	Temperature
Yeast	320	None	...	..	..
Sponge flour	7,500	5,100	...	..	..
Dough flour	3,200	1,800	...	..	..
	Normal trough				
					°C.
Sponge set	20,100	17,500	6,200,000	6.0	22
Sponge remix	29,800,000	11,500,000	45,700,000	4.8	28
Dough set	26,500,000	9,800,000	40,600,000	6.6	28
Dough to the machine	25,400,000	9,000,000	38,300,000	6.5	29
Cracker pH				7.2	
	Sterile trough				
Sponge set	22,000	18,000	6,500,000	6.0	22
Sponge remix	17,200,000	2,800,000	24,200,000	5.2	27
Dough set	15,500,000	2,000,000	21,500,000	6.9	28
Dough to the machine	14,100,000	1,500,000	19,700,000	6.8	28
Cracker pH				8.8	

It is observed from commercial practice that the range of quantity of yeast used in cracker doughs, at which flavor, tenderness, and oven-spring of the cracker are satisfactory, is quite large (from 0.20 to 0.50%). However, uniformity in fermentation and cracker can be better maintained at yeast levels up to 0.50%. At such levels the cracker has better oven-spring and is more tender. At levels over 0.50%, acid-producing bacteria are retarded to such a degree that the cracker has an abnormal pH rise upon baking and a high pH in the finished product (see Fig. 1), with an undesirable flavor.

Sterile Trough. If new or sterile troughs are used, fermentation is greatly retarded, as is shown in Table VI. In the sponge at remix, the bacterial and yeast counts are less for the sterile than for the normal trough. The acid-producing bacterial count at sponge remix is much less for the sterile than for the normal trough. At the same time, the acidity of the sponge in the sterile trough is so much less that there is an abnormal rise in pH and consequently the finished cracker has a high pH (8.8) and an undesirable flavor. This shows that the condition of the sponge fermentation trough with respect to its acid-producer population, due to dough from previous batches adhering to the trough, is an even more important factor in determining bacterial fermentation for flavor and pH response in crackers than is the bacterial flora of the flour.

Literature Cited

1. American Public Health Association. Standard methods for the examination of dairy products, microbiological, bioassay and chemical (8th ed.). The Association: New York, N. Y. (1941).
2. Beccard, E. Beiträge zur Kenntnis der Sauerteiggärung. Centralblatt Bakt. Parasitenk. II, **54**: 465–471 (1921).
3. Kent-Jones, D. W., and Amos, A. J. Modern cereal chemistry. Northern Publishing Co., Ltd.: Liverpool, England (1947).
4. Micka, J. Lactic fermentation and trace iron affect cracker quality. Food Ind. **20**: 102–107, 234 (1948).

THE EFFECT OF TEMPERATURE ON THE RATE OF PENETRATION OF MOISTURE WITHIN DAMPED WHEAT GRAINS[1]

J. D. CAMPBELL AND C. R. JONES

ABSTRACT

The rate of penetration of moisture to the cheek centers of damped Manitoba wheat grains is increased threefold by a rise of 12°C. between 20° and 43.5°C. The periods required for 85% completion of moisture movement at 20°, 31.5°, and 43.5°C. are 24, 8, and 2.6 hours respectively. In this temperature range the rate of change of the moisture diffusion coefficient with temperature appears to be 50% greater than that of the vapor pressure of water, but above 43.5°C. the rate falls off rapidly and there is no change between 49° and 60°C. The observations relate only to grains remaining vitreous after exposure to warmth while damp; the proportion of these grains decreases with increasing temperature and duration of treatment. Initial warming of damped grain to 43.5°C. for periods of 1 hour or more greatly shortens the subsequent period at 20°C. required for completion of moisture distribution.

The penetration of water into wheat immersed at different temperatures has been studied previously (1), but the results do not bear directly on milling practice where wheat is conditioned by moistening to the extent of only about 5%, the added moisture being allowed to penetrate the interior of individual grains during a period of storage. A new method, depending on microdetermination of endosperm density, was recently used to follow the rate of penetration into the cheek centers of damped Manitoba grains at 20°C. (3). Comparable results for different temperatures are now presented.

Materials and Methods

Wheat. The sample of No. 1 Manitoba Northern wheat, previously described (3) as "Manitoba A," was mainly used but similar results were obtained on vitreous grains from other lots of Manitoba wheat.

Moisture Content and Density Determinations were made as previously described (3).

Adjustment of Moisture Content and Temperature. In order to ensure uniform damping, the wheat was graded in grain size and wetted by immersion for 1 minute, followed by centrifuging, as previously described (3). The aim was to obtain grains at 17.2% moisture content to match the samples previously tested for varying periods at 20°C. (3, Fig. 4). Slight accidental departures from this moisture con-

[1] Manuscript received November 30, 1954. Contribution from the Research Association of British Flour Millers, St. Albans, England.

tent hardly affect the steep initial part of the curve for rate of penetration, since here they are small in comparison with the general experimental uncertainty, but where the samples related to the flatter part of the curve (corresponding to more than half the total density change) such departures were compensated for by making an appropriate adjustment, read from Fig. 3 of reference 3 – the graph relating density and moisture content – to the recorded density value. The justification for this procedure is that experiments (which need not be cited fully in this paper) have shown that the flatter portions of the curves for rate of penetration are substantially parallel for grains damped to slightly different extents.

The main practical difficulty in a study of moisture distribution in grain at an elevated temperature is to avoid migration of moisture induced by temperature gradients set up during cooling at the end of the warm period. It is common experience that when a stoppered jar containing warm wheat is cooled, the inner wall of the jar becomes coated with moisture distilled from the wheat. The difficulty was overcome by placing the damped grain under oil (medicinal liquid paraffin) and letting the oil serve as the vehicle conveying heat, a) to warm the grain initially, b) to maintain its temperature for the required period, and c) to cool it prior to dissection (under oil) in the manner previously described (3). Preliminary experiments showed that at moderate temperatures the oil acted as an effective sealing agent against moisture loss from individual wheat grains or endosperm particles. Admittedly, as is well known, moist granular material may be readily dried by being heated under oil. It was found, however, that only at temperatures above 60°C. was there any appreciable disturbance due to translocation of moisture from endosperm to oil as shown by a rise in density of the immersed particle. Furthermore, at moderate temperatures the contact of endosperm with oil, even if prolonged, did not itself affect the endosperm density. Thus substantially similar values for density of endosperm from the cheek centers of wheat grains at 17% moisture content were obtained, irrespective of whether the grains had been

a) stored 3 days under oil at 45°C., b) stored 3 days (without oil) in a stoppered jar at 45°C., then covered with oil prior to cooling, or c) stored 3 days (without oil) in a stoppered jar at room temperature.

Moreover, even when the oil in which the grains were immersed was cooled rapidly from 45°C. to room temperature (18°C.), the value for density of central cheek endosperm was the same as that for grain stored (without oil) at room temperature. Evidently, no migration of

moisture within individual oil-sealed grains occurs during cooling.

In general, when initially vitreous grains of Manitoba wheat are damped to about 17% moisture content, warmed, and subsequently cooled, some of the grains show mealiness of endosperm. The proportion of mealy grains appears to increase with increasing temperature and with increasing period of heating at any given temperature, but the present results relate only to those grains that retained their vitreous character after treatment. At higher temperatures and after longer periods of treatment such grains formed a minority – in the extreme case, less than 20% of the total number of grains treated.

In the previous communications (3, p. 184) it was mentioned that, beyond a certain degree, mealiness of endosperm is associated with an appreciable lowering of density. The extent of this lowering appears to be increased after damped grain is warmed. It is then also possible for some of the grains that have retained a vitreous appearance to show a slight fall in density of endosperm. This follows from the observation that the spread of values, for density of central cheek endosperm, between vitreous grains of a given sample increases progressively with severity of heat treatment. The maximum values remain substantially unchanged but the minimum values become progressively lower. Thus the following figures show the differences, expressed as units in the third decimal place, between the unchanged maximum value and the minimum values, after different periods of warming at 31.5°C. Each figure is based on the values, for density of central cheek endosperm, found in a set of six vitreous grains from a sample of Manitoba wheat at 17.2% moisture content warmed for the period in question.

Period (hours)	:	2	4	7	26
Difference	:	0.8	2.0	2.9	3.7

However, irrespective of the duration of the period of heat treatment, a substantial proportion of the grains remaining vitreous show density values close to the maximum; i.e., frequency curves (not reproduced here) show marked asymmetry, with a well-defined mode corresponding to a small class interval (usually 0.001) at the upper end of the range. The uppermost value in this class interval was used in marking each point for warm wheat in the accompanying figures. Usually endosperm particles from six vitreous grains were examined in fixing each point and the values for half the number of grains fell within the class interval specified.

Results and Discussion

Figure 1 shows results for the temperature range 20°–43.5°C. (68°–110°F.), the curve for 20°C. being reproduced from Fig. 4 in

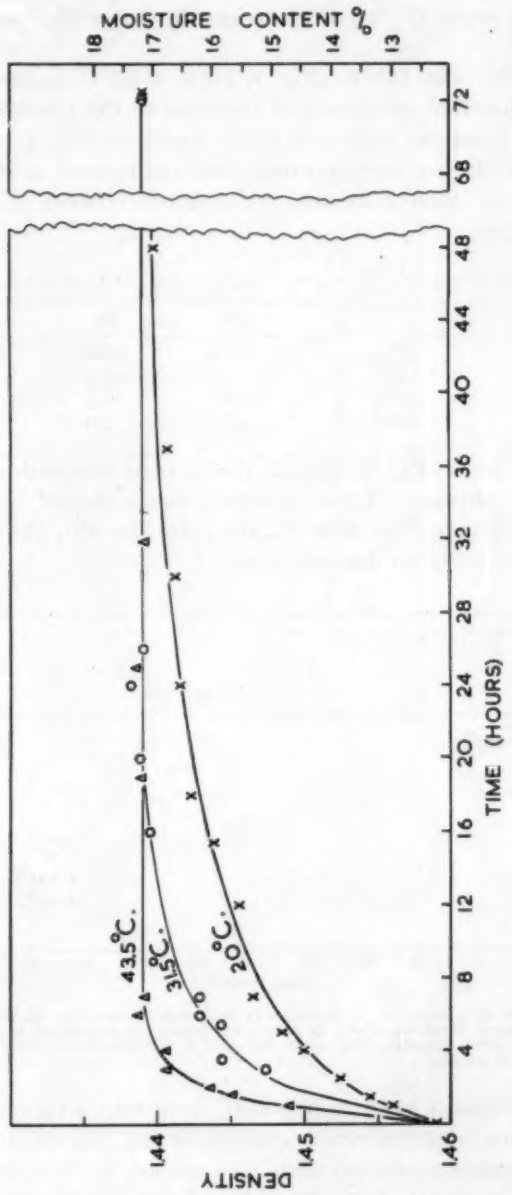


Fig. 1. Rates of penetration of moisture at three different temperatures up to 43.5°C. into damped Manitoba wheat as shown by density of endosperm in center of cheek at various times after damping.

the previous paper (3). Figure 2 covers the range 43.5°–60°C. (110°–140°F.).

Between 20° and 43.5°C. (Fig. 1) a rise of 12°C. causes a threefold increase in speed of movement of moisture to the cheek center. This is apparent from the following table, based on Fig. 1, showing the periods, at different temperatures, that correspond to various proportions of the final increment in moisture content of the central cheek endosperm:

Temperature		*Percentage of final increment*		
		50	85	100
°C.	*°F.*	*hours*	*hours*	*hours*
20.0	68	5	24.0	60
31.5	89	2	8.0	20
43.5	110	<1	2.6	6

However, above 43.5°C. (Fig. 2) the increase in speed of movement ceases rather abruptly. There is only a slight change from 43.5° to 49°C. (120°F.), then none to 60°C., the points for 49°, 54.5°, and 60°C. falling substantially on the one curve.

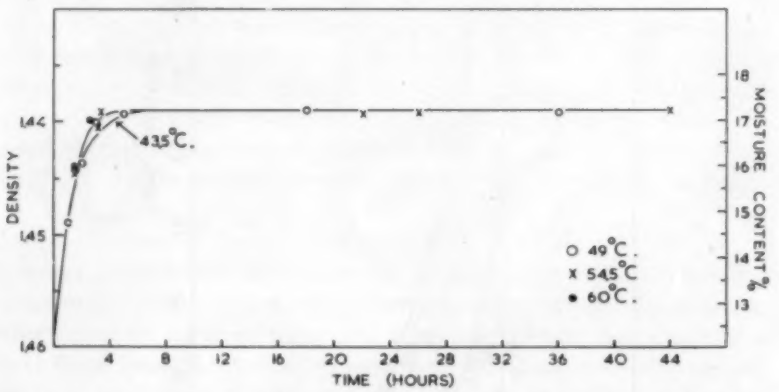


Fig. 2. Rates of penetration of moisture at temperatures between 43.5° and 60°C., inclusive, into damped Manitoba wheat as shown by density of endosperm in center of cheek at various times after damping. The curve for 43.5°C. is reproduced from Fig. 1 without showing individual points.

It has previously been deduced (2), from consideration of data on rates of drying, that for wheat endosperm the rate of change of the moisture-diffusion coefficient with temperature was similar to that of the vapor pressure of water. The rate, up to 43°C. from the present data, is roughly 50% greater than this. The drying data cited related, however, to the grain as a whole and the deduction presupposed

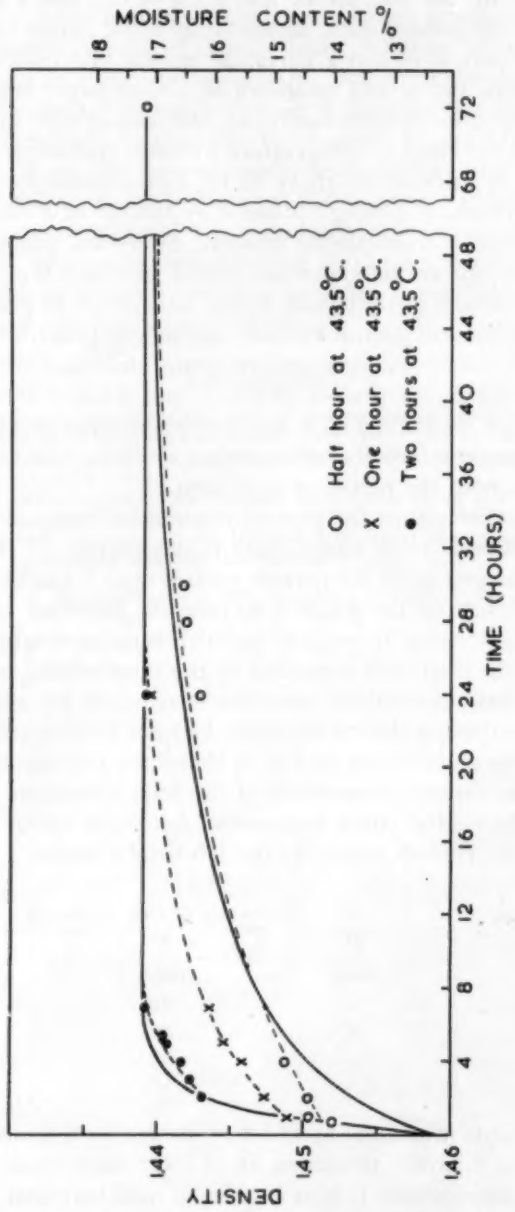


Fig. 3. Rates of penetration of moisture at 20°C. into damped Manitoba wheat, following initial warming to 43.5°C. for the periods shown. The continuous lines reproduce the curves for 20° and 43.5°C. from Fig. 1 without showing individual points.

homogeneity of the interior of the grain with respect to moisture movement. The present data, on the other hand, relate to movement between the periphery and a particular region (the central cheek) of the endosperm, and it will be shown in a later paper that the speed of movement into different parts of the endosperm varies. Fuller discussion of the effect of temperature increase, including the remarkable feature of its cessation above 43°C., will accordingly be reserved.

Effect of Initial Warming Followed by Storage at Room Temperature. In European conditioning practice, Manitoba wheat is warmed to 43.5°–49°C. for an hour or more, cooled and then bin-stored, often for 24 hours, before being milled. It was accordingly of practical interest to use the present technic to study the rate of penetration at 20°C. (representing room temperature) in grain that initially had been warmed to 43.5°C. for periods of 0.5, 1, and 2 hours after damping. The results are shown in Fig. 3, the continuous lines of which are the curves previously shown for temperatures of 20°C. and 43.5°C. maintained throughout the period of treatment.

It is remarkable that the period required for completion of moisture distribution at room temperature is affected only by initial warming of the damped grain for periods greater than 0.5 hour. With such periods, the effect on the graph is to raise the curve for rate of penetration while keeping it roughly parallel with its original position. Accordingly the final level is reached by the curve while it is still steep; this means that substantially less time is required for completion of moisture distribution than if the grain had not been warmed initially. The following table, based on Fig. 3, shows the periods at 20°C. that correspond to various proportions of the final increment in moisture content of the central cheek endosperm, following initial warming at 43.5°C. for the periods shown in the left-hand column:

Initial period at 43.5°C.	*Percentage of final increment*		
	50	85	100
hours	*hours*	*hours*	*hours*
0.0	5	24	60
0.5	4	26	60
1.0	...	10	28
2.0	...	3	8

Thus 2 hours' treatment at 43.5°C. followed by 6 hours at 20°C. is as effective as 6 hours' treatment at 43.5°C.: both cause completion of moisture distribution. It is of interest to note here that, while early in the active development of warm conditioning practice (during the decade following the first World War) periods of warm treatment of

the order of only 0.5 hour were used, more recent practice (4) has been to employ longer periods, of about 1 hour (followed by cooling and a period—usually 24 hours—of bin storage). It must, however, be emphasized that the period of warm treatment includes the time – at least 20 minutes—required to raise the temperature of the wheat from its initial level. From the present results prolongation of the period of warm treatment to about 2 hours at 43.5°C. would reduce the subsequent bin period required for completion of moisture distribution almost to the minimum corresponding to this temperature of initial treatment. These observations are, however, presented at this stage without direct reference to milling results.

Literature Cited

1. Jones, C. R. Observations on the rate of penetration of water into the wheat grain. Milling (Liverpool) **113**: 80–86 (1949). *Also:* Rate of water penetration into the wheat grain. Northwestern Miller, Milling Production Section **239** (11, Sec. 2): 3a–8a (1949).
2. Jones, C. R. Evaporation in low vacuum from warm granular material (wheat) during the falling-rate period. J. Sci. Food and Agr. **2**: 565–571 (1951).
3. Jones, C. R., and Campbell, J. D. Micro-determination of endosperm density as a means of mapping moisture distribution in wheat grains. Cereal Chem. **30**: 177–189 (1953).
4. Lockwood, J. F. Flour milling (p. 203). Northern Publishing Co. Ltd.: Liverpool (1945).

THE INHIBITION OF WHEAT LIPOXIDASE BY CYANIDE[1]

G. N. IRVINE and J. A. ANDERSON

ABSTRACT

Cyanide concentrations of the order of 5×10^{-3} *M* inhibit crude wheat lipoxidase as much as 63%. The reaction of cyanide with the enzyme is sluggish and maximum inhibition occurs only when addition of substrate is delayed for about 15 minutes. The second (zero order) phase of the reaction appears to be almost completely inhibited by these levels of cyanide. The degree of inhibition depends on the enzyme level and also on the total fluid volume used.

From studies of the rate of oxidation of the xanthophyll pigments of durum semolinas during macaroni processing, it was postulated (4) that durum wheats contained the enzyme lipoxidase. At the same time it was reported that cyanide ion in low concentration inhibited the oxidation in macaroni doughs. Others, working with various crude preparations of lipoxidase, have reported both that lipoxidase was (1, 6) and was not inhibited by cyanide (3). Holman (2), working with crystalline soybean lipoxidase, stated that the crystalline enzyme was not inhibited by cyanide or other common enzyme inhibitors, but was inhibited by antioxidants. Accordingly the question of what constitutes the active group of lipoxidase has remained in doubt.

Following the work on dough, studies of the lipoxidase activity of crude aqueous extracts of wheat were undertaken with a Warburg apparatus. Early in this work the effect of cyanide as an inhibitor of this system was confirmed; but the evidence was not clear-cut; the inhibition became appreciable only at longer reaction times. It has now been found unequivocally that wheat lipoxidase is inhibited by low concentrations of cyanide. The reaction between the enzyme and cyanide is not instantaneous; the two must be equilibrated for about 15 minutes before addition of the substrate in order that the inhibition can be properly observed. This behavior helps to account for the different results previously obtained by other investigators. It may also account for the absence of inhibition in the studies of Holman with the crystalline enzyme; he used a 2-minute assay procedure, and there was thus insufficient time for the inhibitor to act on the enzyme.

[1] Manuscript received December 13, 1954. Paper No. 141 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 319 of the Associate Committee on Grain Research (Canada).

Experimental

The source of the enzyme for the studies reported in this paper was Pelissier durum wheat; aqueous extracts were obtained as outlined previously (5). The substrate was a linoleic acid emulsion (5) containing about 0.02 ml. acid per ml., and the experiments were conducted in a Warburg apparatus at pH 6.5 (phosphate buffer) and a temperature of 30°C.

The cyanide concentration used was 5×10^{-3} *M*. The amount of inhibition observed at this level of cyanide depends upon two factors: the length of time cyanide and enzyme are allowed to equilibrate together before the substrate is added, and the concentration of enzyme employed. To study these two features of the inhibition, 50-ml. Warburg flasks with two sidearms were used. In this way the enzyme could be tipped into the cyanide-buffer when the temperature equilibrium was reached, and the substrate tipped in to start the reaction after a suitable equilibration time. The substrate volume was 1.0 ml., the total fluid volume was 6.0 ml., and the atmosphere in the flasks was air.

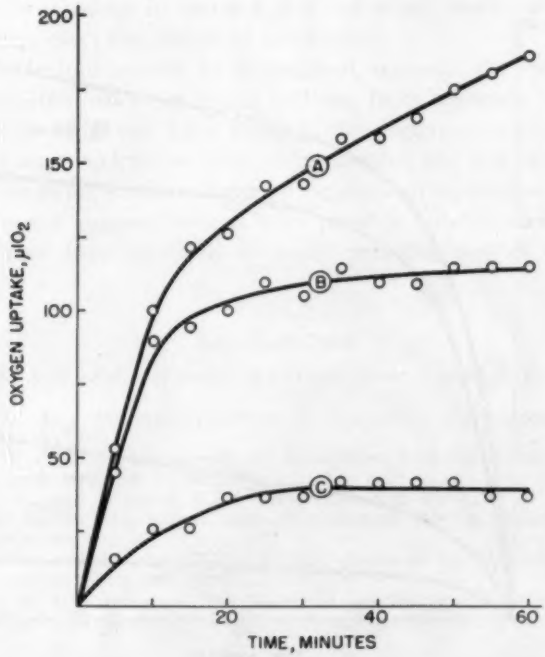


Fig. 1. The effect of time of equilibration of enzyme and cyanide on the degree of inhibition. A, control, no cyanide; B, enzyme, substrate, and cyanide mixed simultaneously; C, enzyme and cyanide equilibrated 15 minutes before addition of substrate.

Figure 1 shows the effect of time of equilibration of cyanide and enzyme on the inhibition. Curve A represents a control without cyanide; curve B represents substrate, enzyme, and cyanide-buffer mixed simultaneously; and curve C represents enzyme and cyanide-buffer equilibrated for 15 minutes before addition of substrate. The enzyme concentration used in this experiment was quite low (0.16 ml. of extract), and under these conditions a slight inhibition is immediately apparent when enzyme, cyanide, and substrate are mixed simultaneously; the most pronounced effect, however, is not apparent until after 15–20 minutes of reaction time. On the other hand, when enzyme and cyanide are allowed 15 minutes to react before the substrate is added, the inhibition is very marked from the beginning of the reaction. The biphasic nature of the lipoxidase reaction has been discussed previously (5), and it is interesting to note that cyanide almost completely inhibits the steady state, zero order phase of the reaction.

Curves illustrating inhibition at enzyme concentrations obtained with 0.16 ml. and 0.8 ml. of extract, at a fixed fluid volume (6.0 ml.) and cyanide concentration (0.005 *M*) are shown in Fig. 2; in each case

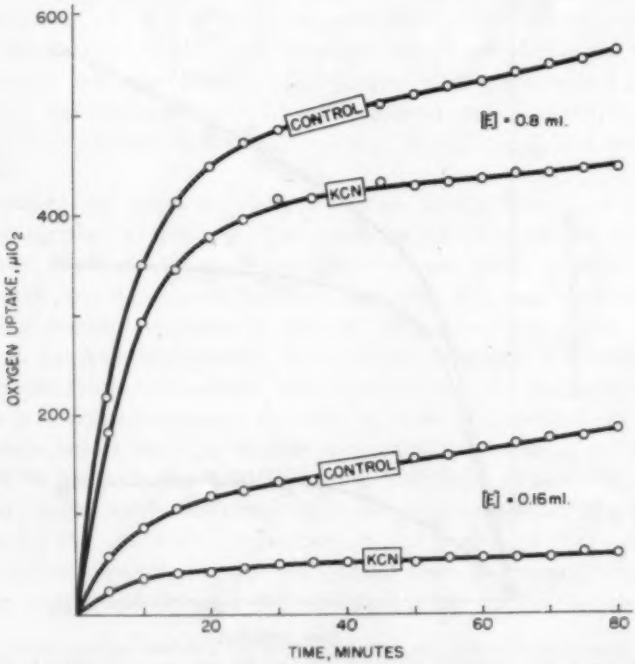


Fig. 2. Effect of enzyme concentration on degree of inhibition by 0.005 M potassium cyanide solution.

the enzyme and cyanide were given 15 minutes to react before the substrate was introduced. Enzyme concentration has a very pronounced effect on the degree of inhibition. These results are typical; the percentages of inhibition as measured by the 5-minute uptake, observed for a wider series of enzyme concentrations, were as follows:

Enzyme Extract Used ml.	*Inhibition* %
0.80	16
.48	22
.32	52
.16	63
0.08	62

Discussion

The main conclusions drawn from this study are that crude wheat lipoxidase is strongly inhibited by cyanide, but that the reaction between cyanide and enzyme is sluggish. Adequate equilibration of enzyme and cyanide is required, before the substrate is added, in order to demonstrate inhibition. Other unpublished studies have also shown that variations in enzyme and substrate levels, and in total fluid volume, affect the degree of inhibition.

The conclusions cannot be generalized, because: the enzyme preparation was obtained from wheat and not from soybeans, with which most other investigators have worked; the preparation was not purified in any way; and the activity measurement and pH differed from those used in prior studies of crystalline soybean lipoxidase. Nevertheless, the results suggest reasons why possible inhibition by cyanide may not have been detected in some previous studies of soybean lipoxidase.

Literature Cited

1. Craig, F. N. A fat oxidation system in *Lupinus albus*. J. Biol. Chem. **114:** 727–746 (1936).
2. Holman, R. T. Crystalline lipoxidase. II. Lipoxidase activity. Arch. Biochem. **15:** 403–413 (1947).
3. Hummel, J. P., and Mattill, H. A. Lipoxidase, a dehydrogenase. Proc. Soc. Exptl. Biol. Med. **55:** 31–33 (1944).
4. Irvine, G. N., and Winkler, C. A. Factors affecting the color of macaroni. II. Kinetic studies of pigment destruction during mixing. Cereal Chem. **27:** 205–218 (1950).
5. Irvine, G. N., and Anderson, J. A. Kinetic studies of the lipoxidase system of wheat. Cereal Chem. **30:** 247–255 (1953).
6. Süllmann, H. Die Aufnahme von Sauerstoff bei der enzymatischen Oxydation ungesättigter Fettsäuren. Helv. chim. Acta **24:** 1360–1380 (1941).

VISCOSITY vs. PROTEIN AND ASH CONTENT OF WESTERN WHEAT VARIETIES[1]

C. R. Bresson and M. A. Barmore[2]

ABSTRACT

Highly significant positive correlations (0.70 to 0.95) between flour viscosity and flour protein content have been obtained for 17 commercial varieties common to the Pacific Northwest. Regressions are linear and as a group are fan-shaped, with a common origin or nearly so. The hard wheats suitable for making bread have the greatest slope and the soft wheats suitable for pastry flours the least, with the intermediate or dual-purpose types in between.

The slopes of the viscosity-protein regression lines are highly correlated with the constants of their regression equations. This makes it possible to calculate the best average point of intersection of the 17 variety-protein viscosity regression lines. A family of lines can be drawn through this point to form an idealized chart representing the effect of protein content on viscosity for various types of flour. By means of this chart the flour viscosity data of plant breeders' wheat hybrids can be compared with well-known commercial varieties and with each other on a comparable protein basis. This provides one of the best single tests available for classifying the quality of soft and semihard western varieties.

Flour ash decreased the viscosity significantly in 60% of the varieties, but the constants of the regression equations do not seem to be correlated with any other known character of the flour. Consequently, there is no apparent method of generalizing this information so that a correction factor for ash can be applied to new and unknown varieties.

The method of determining the "apparent viscosity of acidulated flour-water suspensions," based on the work of Sharp and Gortner (14) and described in *Cereal Laboratory Methods* (1), is widely used by the soft wheat milling industry. It was developed to its present form by Blish and Sandstedt (6), Vogel and Bailey (15), Johnson and Herrington (9), Larmour and Sallans (10), Bayfield (2, 3, 4, 5), Reiman (12), and Rich (13).

Most of these investigators observed that viscosity increased with the flour protein content. Some of them proposed methods to eliminate or correct for the effects as follows: by using the relation of log of viscosity to log of flour concentration (8); by using the amount of flour containing 2 g. of protein (4, 12, 13); by dividing viscosity readings by the amount of protein in the flour used (6); or by diluting the flour samples with starch to a common protein level (5). Although Sharp and Gortner's (14) investigation suggests that viscosity is a linear

[1] Manuscript received October 7, 1954. Contribution from the Western Wheat Quality Laboratory, Department of Agricultural Chemistry, Washington Agricultural Experiment Stations, State College of Washington, and Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture. Scientific Paper No. 1355, Washington Agricultural Experiment Stations, Pullman.

[2] Baking Technologist and Senior Chemist, Field Crops Research Branch, A.R.S.

function of protein content, Larmour and Sallans (10) evidently were the first to point it out. They worked only with blends of flour from the hard red spring variety Marquis.

Unpublished reports by Morris *et al.* (11) show that the effect of protein content was linear for 11 eastern soft red winter varieties, and that an idealized fan-shaped group of lines could be fitted to these data. Of these several proposals, only the method employing 2 g. protein is mentioned in *Cereal Laboratory Methods* (1) as a means of eliminating the effect of varying flour protein content.

Considerable effort has been expended by many workers (6, 8, 9, 10, 15) to eliminate variations in viscosity due to ash content of flour, since increases in flour ash were found to reduce viscosity markedly. No satisfactory method of eliminating or correcting for the effect of variations in flour ash has been published.

During the course of the work of characterizing new wheat varieties and comparing them with established current commercial varieties, this laboratory has assembled considerable data on flour viscosity as related to protein and ash content. The data for 17 commercial varieties common to the Pacific Northwest have been summarized by the calculation of correlation coefficients and regression equations which are presented in this paper.

Materials and Methods

The samples were obtained from State Experiment Station plots in Washington, Oregon, Idaho, Utah, and western Montana over the past 6 years. The grain was all sound, of 58 lb. or higher test weight, and experimentally milled on a Buhler mill. Protein content of the flour ranged from 4.9 to 14.7% with a mean of 9.0%; ash content varied from 0.27 to 0.50%, with a mean of 0.39%.

The viscosity determinations were made as directed in *Cereal Laboratory Methods*, except that there was no digestion period and there was only one addition of 7 ml. of 1 *N* lactic acid. This method was adopted after examination of the results of 100 samples run by the A.A.C.C. increment method. In practically all cases, the maximum viscosity was obtained with 7 ml. of acid. Very few samples reached a maximum at 5 ml., and in these the viscosity values were not appreciably different from the viscosity obtained on the addition of 2 ml. more acid.

Results and Discussion

Table I gives the number of samples of each variety on which viscosity determinations were made, the regression equation (column

TABLE I

STATISTICAL DATA FOR VISCOSITY VS. FLOUR PROTEIN AND ASH

Variety	No. of Samples	Viscosity vs. Protein		Viscosity vs. Protein–Ash	
		Regression Equation[a]	Correlation Coefficient	Regression Equation[a]	Multiple Correlation Coefficient
Kharkof	18	V = 28.11 P − 136.61	0.86**	V = 27.6 P − 89 A − 95	0.86
Rio	41	V = 28.05 P − 131.50	.89**	V = 26.4 P − 272 A − 1	.90*[b]
Wasatch	17	V = 22.47 P − 111.60	.77**	V = 19.8 P − 930 A + 319	.86*
Orfed	54	V = 20.45 P − 100.30	.91**	V = 20.7 P − 221 A − 25	.93**
Triplet	33	V = 20.45 P − 93.56	.90**	V = 20.6 P − 90 A − 64	.90
Marfed	49	V = 17.60 P − 78.46	.83**	V = 21.1 P − 287 A + 3	.95**
Rex	51	V = 16.62 P − 79.99	.95**	V = 17.1 P − 175 A − 14	.97**
Lemhi	19	V = 16.48 P − 88.35	.95**	V = 16.2 P − 72 A − 57	.95
Idaed	36	V = 15.79 P − 62.87	.87**	V = 16.9 P − 54 A − 55	.92**
Awned Onas	18	V = 14.94 P − 73.74	.87**	V = 14.9 P − 148 A − 13	.89**
Hymar	32	V = 13.82 P − 60.61	.94**	V = 14.0 P − 93 A − 24	.95*
Baart	41	V = 13.69 P − 52.29	.70**	V = 13.5 P − 149 A + 12	.78
Federation	39	V = 12.03 P − 51.70	.90**	V = 12.3 P − 186 A + 20	.93**
Brevor	27	V = 10.54 P − 31.98	.88**	V = 10.7 P − 147 A + 21	.94**
Golden	41	V = 8.95 P − 38.17	.89**	V = 9.0 P − 10 A − 35	.89
Elgin	72	V = 5.42 P − 10.82	.84**	V = 5.2 P + 26 A − 20	.84
Elmar	28	V = 5.13 P − 9.44	0.88**	V = 5.3 P − 53 A + 11	0.89

*Significant at 5% point. **Significant at 1% point.

[a] V = Viscosity; P = flour protein (%); A = flour ash (%).

[b] In this column the * indicates a significant and the ** a highly significant improvement in the viscosity prediction due to the inclusion of A with P in the equation.

3), and the correlation coefficients (column 4) for the relation between viscosity and flour protein content. These coefficients are all highly significant. The right half of the table (columns 5 and 6) gives the multiple correlation coefficient and the regression equation for viscosity, protein, and ash. To give a more graphic picture of the variation, the data on viscosity and flour protein for three varieties have been plotted in Fig. 1. The varieties are: Elgin, a soft white club; Rex, a soft white common type; and Rio, a hard red variety very similar to Turkey.

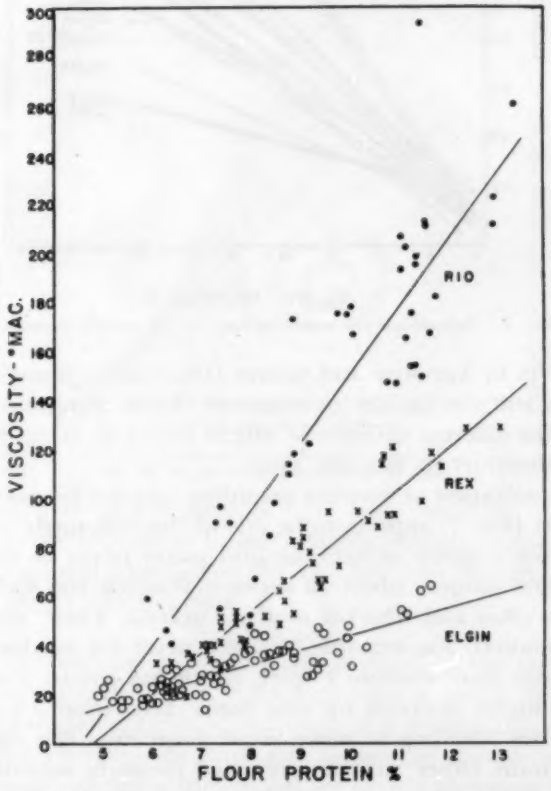


Fig. 1. Distribution of viscosity-protein data for three typical varieties.

Figure 2 illustrates the relative locations of the regression lines of each variety. These lines are typically fan-shaped, apparently intersecting approximately at a common point. This linearity of the protein-viscosity relationship has been pointed out for a single hard spring

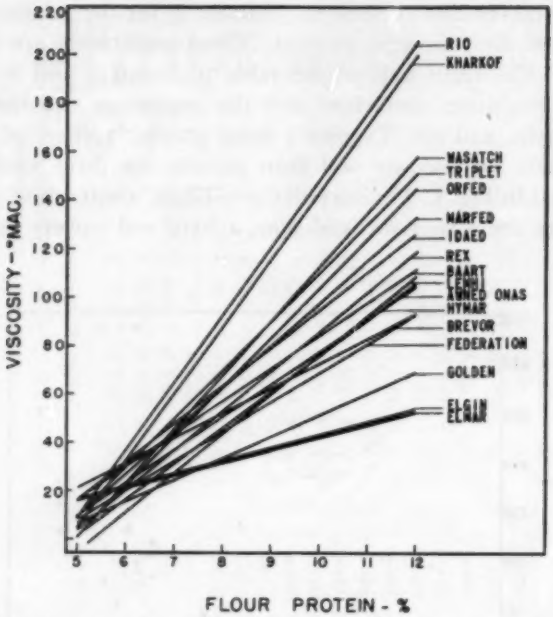


Fig. 2. Viscosity-protein regression lines of 17 western varieties.

wheat variety by Larmour and Sallans (10). Similar linearity for soft red wheats and the fanlike arrangement of the regression lines was shown for the different varieties by Morris *et al.* (11) at the Soft Wheat Quality Laboratory in Wooster, Ohio.

This classification of varieties according to their viscosity and protein content (Fig. 2) appears to be one of the best single methods of characterizing a group of varieties into pastry (those at or near the bottom), dual purpose (those in between Wasatch and Golden), and bread types (Rio and Kharkof or the Turkeys). There are some exceptions, however. For example, Wasatch never has produced a satisfactory pastry flour whereas Triplet, which has similar viscosities, is considered highly desirable for cake flours. Federation is a much better variety for blending to make bread flours than this classification would indicate. Other varieties are rated correctly according to the present available information relative to their use by the milling industry of the Pacific Northwest.

Morris *et al.* (11) constructed a chart with a fan-shaped series of lines to represent an idealized relationship of viscosity and protein content very similar to Fig. 3 to represent the data and to be used in correcting viscosity values of unknown varieties to a uniform pro-

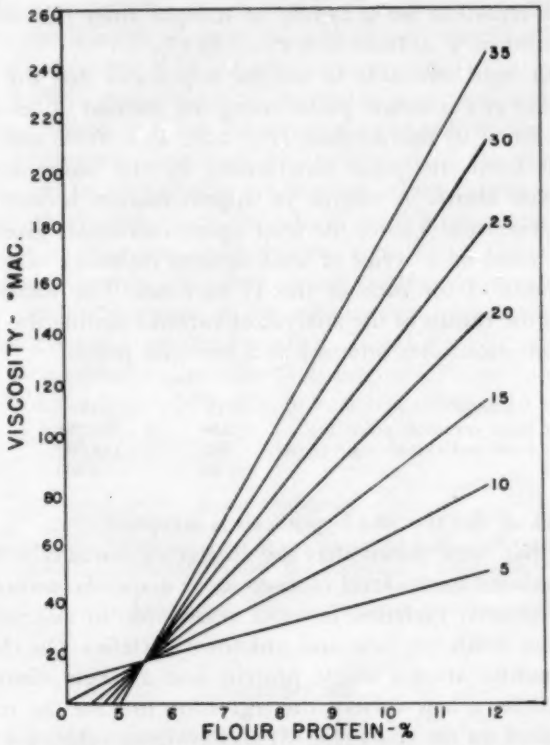


Fig. 3. Idealized chart of viscosity-protein relationship at various levels, to be used for correcting viscosity values for differences in protein content of flour.

tein content. The lines appear to have been fitted by observation alone. A sounder method of fitting a family of lines to these data is to write the general equation of the viscosity-protein regression lines for each of the 17 varieties thus:

$$V = bP - C \qquad (1)$$

where V is the viscosity in degrees MacMichael, b the slope of the regression line for each variety, P the flour protein, and C the constant for each variety. From Table I, column 3, it is evident that there is a close association between the slope (the amount of change in viscosity per unit change in protein content) of the simple regression lines and the constant of the equation. The correlation coefficient between these factors was found to be -0.986 and the regression equation was

$$C = -5.52\,b + 16.53 \qquad (2)$$

Substitution of equation 2 for C in equation 1 and rearranging gives

$$V - 16.53 = b\,(P - 5.52) \qquad (3)$$

This is the equation for a family of straight lines intersecting at a common point of $V = 16.53$ and $P = 5.52$ (7).

It was thought advisable to test the hypothesis that the regression lines intersect at a common point, using the method of least squares.[3] The point found by this method ($P = 5.72$, $V = 20.26$) was not greatly different from the point determined by the more approximate method given above. A degree of approximation is introduced in calculating equation 3 since the least squares estimates given in equation 2 are based on a series of least squares estimates obtained from fitting equation 1 for each of the 17 varieties. The following table summarizes the results of the analysis of variance testing the hypothesis that the regression lines intersect at a common point.

Source	*df*	*SS*	*MS*
Deviation from common point reg.	598	150,796	
Deviation from individual reg. (error)	582	146,396	251.54
Difference	16	4,400	275.03

On the basis of this test, the hypothesis is accepted.

Since it has been shown that the change of viscosity with protein content is related to the level of viscosity at a specific protein content for the 17 western varieties, it seems reasonable to assume that this same relation holds for new and unknown varieties. On the basis of this relationship, after a single protein and a single viscosity value are obtained for a new variety, the regression line for the new variety may be located on the chart (Fig. 3) and viscosity values for any other protein values may be predicted provided they are determined by the described method.

The chart used by Morris *et al.* (11) was obtained from the protein-viscosity regression lines of samples of 11 soft wheat varieties grown during a 5-year period at Experiment Stations in Michigan, Illinois, Kentucky, Ohio, Oklahoma, and New York. Viscosities were determined by the same method except that 6 ml. instead of 7 ml. of lactic acid were added at one time. The family of lines calculated by the above method is represented by

$$V - 18.40 = b\,(P - 5.65)$$

This is almost exactly the same as the equation representing the data obtained from the western varieties by a slightly different viscosity method. Evidently the same correction factors, for differences in protein content, can be applied to both groups of varieties in both laboratories. It may be more widely adaptable but should not be applied in other laboratories without proof of its applicability.

[3] W. D. Hanson, private communication, August 26, 1954.

Since the literature has shown that flour ash affects viscosity values, the data on the western varieties were examined for the effect of this factor as well. The result of introducing flour ash (A) as a second independent variate in the multiple regression equation is given in the last two columns of Table I. The inclusion of ash in the prediction equation significantly improved the prediction of viscosity in 11 of the 17 varieties. In all varieties an increase in ash caused a reduction in viscosity, except for Elgin, in which the viscosity was increased. However, the increase was small and not significant. There seemed to be no relation between the effect of ash content on viscosity (the A coefficient) and the constant of the regression equations or any other known characteristics of the varieties. Thus there is no apparent method of developing a generalized viscosity correction factor for the ash content of the flour applicable to varieties other than those listed as significant in Table I.

Acknowledgment

The calculating and interpretation of the statistical portion of this study would have been practically impossible without the cooperative assistance of the Experiment Stations Statistician, Mr. J. G. Darroch, and personnel of the Washington State College Computing Laboratory. The authors wish to acknowledge their debt to these individuals.

Literature Cited

1. American Association of Cereal Chemists. Cereal laboratory methods (5th ed.). The Association: St. Paul, Minnesota (1947).
2. Bayfield, E. G. The viscosity test. Cereal Chem. **11**: 637–647 (1934).
3. Bayfield, E. G. Soft winter wheat studies. II. Evaluating experimentally milled flours with the aid of viscosity, fermentation, and baking tests. Cereal Chem. **11**: 121–140 (1934).
4. Bayfield, E. G. Soft winter wheat studies. III. The effect of some factors influencing viscosity and protein. Cereal Chem. **12**: 1–16 (1935).
5. Bayfield, E. G. (Chmn.) Report of the subcommittee on viscosity. Cereal Chem. **13**: 773–788 (1936).
6. Blish, M. J., and Sandstedt, R. M. Viscosity studies with Nebraska wheat flours. Cereal Chem. **2**: 191–201 (1925).
7. Dull, R. W., and Dull, R. Mathematics for engineers (3rd ed.), p. 402. McGraw Hill: New York (1951).
8. Gortner, R. A. Viscosity as a measure of gluten quality. Cereal Chem. **1**: 75–81 (1924).
9. Johnson, A. H., and Herrington, B. L. Wheat and flour studies. XII. Factors influencing the viscosity of flour-water suspensions. II. Effect of hydrogen-ion concentration during extraction or digestion period. Cereal Chem. **5**: 14–44 (1928).
10. Larmour, R. K., and Sallans, H. R. A study of various measures of viscosity of flour water suspension in relation to quality. Can. J. Research **8**: 364–374 (1933).
11. Morris, V. H., Finney, K. F., Barmore, M. A., Bode, C. E., Heizer, H. K., and Yamazaki, W. T. Seventh annual report of the Federal Soft Wheat Laboratory, 1943 crop. U.S. Dept. Agr., Agr. Research Admin., Bur. Plant Indus., Soils, and Agr. Eng. 33cc, (33 pp., processed) (1945).

12. REIMAN, W. Methods for determining the viscosity of flour-in-water suspensions. Cereal Chem. 11: 299–312 (1934).
13. RICH, C. E. Some observations on the differentiation of cake and pastry flours by the viscosity of their water suspensions. Cereal Chem. 12: 555–558 (1935).
14. SHARP, P. F., and GORTNER, R. A. Viscosity as a measure of hydration capacity of wheat flour and its relation to baking strength. Minn. Agr. Exp. Sta. Tech. Bull. 19 (1923).
15. VOGEL, H., and BAILEY, C. H. A study of durum wheats. Cereal Chem. 4: 136–149 (1927).

GAS PRESSURE IN FERMENTED DOUGHS[1]

C. H. BAILEY[2]

ABSTRACT

When the gas of fermentation is released from a fermented bread dough at the time it is ready for baking, it becomes evident that the gas is under pressure in the vesicles of the dough. In a common type of pan bread dough made with hard wheat flour containing 11.3% of crude protein, the measured pressure of the gas in the vesicles averaged 1.032 atmospheres.

It is evident that the carbon dioxide produced by fermentation in yeast-leavened wheat flour doughs, which effects the expansion in volume of such doughs, must be under some pressure in the vesicles of the dough. So far as the writer is aware, the magnitude of this pressure has never been measured quantitatively. The device for such measurement, employed in these studies, was designed to be as simple as possible and thus capable of ready duplication in the event that supplementary measurements of this nature are conducted elsewhere.

Materials and Methods

The gas-pressure measuring device used in these studies is shown in cross-section in Fig. 1. It comprises a 1-pt. Mason jar with a special lid which, in turn, is fitted with three tubular outlets. The outlet at the right leads through rubber pressure tubing to a glass stopcock. This can be connected to a buret system so manipulated as to measure (a) the increasing volume of the expanding dough in essentially the same manner as described by Bailey and Johnson (1), or (b) the displacement of the deflated dough at the close of the sequence of measurements. The tubular outlet at the left is attached directly to a simple water manometer. In the center of the special Mason-jar lid is a gas-tight tubulare fitted with a movable plunger of metal. At the lower

[1] Manuscript received September 30, 1954. This paper is based upon researches conducted at the Research Laboratories of General Mills, Inc., Minneapolis, Minnesota.
[2] Dean Emeritus, Institute of Agriculture, University of Minnesota.

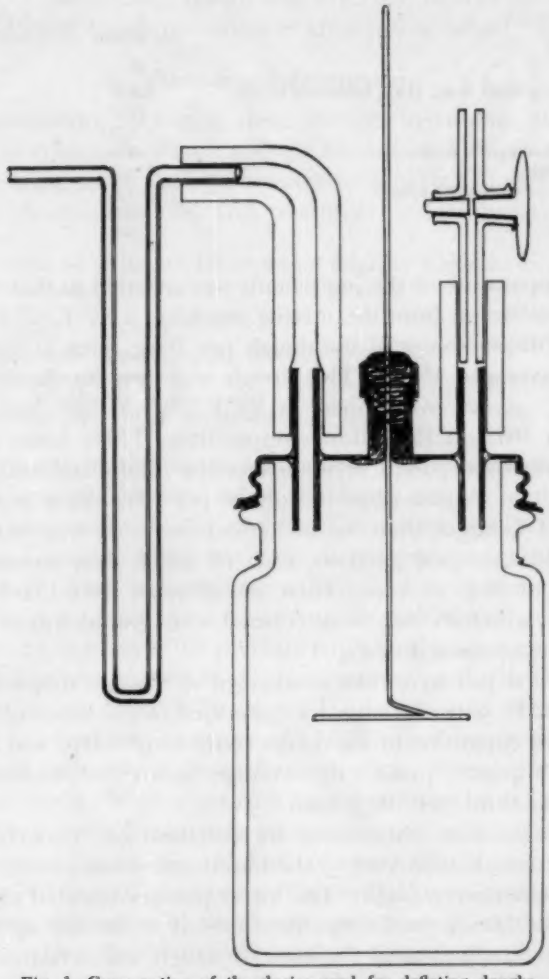


Fig. 1. Cross-section of the device used for deflating doughs.

end of this device is a ring of stainless-steel rod, 3/32 in. in diameter, terminating at the top in a straight rod of the same material which passes out at the top through the tubulare. The latter is stuffed with several wrappings of heavy cord that function as a gas-tight gasket around the movable plunger rod. The operation of this plunger will be described later.

The doughs involved in these studies were all made from the same lot of baker's patent flour, which contained approximately 11.3% protein. The formula of the doughs was as follows:

	As Mixed	*Equivalent of 100 g. Flour*
	g.	*g.*
Baker's patent flour (14% moisture basis)	300.0	100.0
Sugar	15.0	5.0
Salt	6.0	2.0
Nonfat dry milk solids	9.0	3.0
Shortening	6.0	2.0
Compressed baker's yeast	7.5	2.5
	ml.	*ml.*
Water	188	62.7

The temperature of the ingredients was adjusted so that the dough would be delivered from the mixing machine at 82°F. (27.8°C.). The volume or displacement of the dough per 100 g. flour as measured at this stage averaged 150 ml. This dough was then incubated in a fermentation cabinet maintained at 86°F. (30°C.), and was punched twice, after 105 and 150 minutes respectively. Three hours after mixing, the dough was passed between sheeting rollers and molded as for the baking pan. At this stage its volume per 100 g. flour averaged 193 ml., or 29.7% larger than before fermentation. It was weighed and divided into six equal portions, each of which thus represented the equivalent of 50 g. of flour. These six aliquots were placed in 1-pt. Mason jars, which in turn were covered with special lids as described above and diagrammed in Fig. 1.

The several jars were then submerged in a water thermostat maintained at 86°F., with the stopcock connected to the buret of a gasometer. Thus the expansion of the dough could be observed and measured. After 70 minutes' "proof" the average volume of numerous such doughs was 535 ml. per 100 g. flour.

The doughs were then ready for deflation or "knocking down." First, the stopcock connected to the buret system was closed, rendering the dough chamber gas-tight. The metal plunger inserted through the center of the Mason jar lid was then moved vigorously up and down about 10 or 12 times until the mass of dough was detached from the sides and bottom of the jar and balled up around the lower ring of the plunger. During this deflating process the pressure of gases in the closed system mounted, and this rise of pressure in mm. of water was read on a millimeter scale held behind the water manometer. In order to determine whether the system was gas-tight, it was permitted to stand undisturbed for 15–20 seconds after the deflating agitation ceased, and the manometer was observed to note whether the upper level receded. In the few cases where this occurred, indicating some leakage of gases, the observations were not recorded. Generally the system proved to be gas-tight. Under these conditions the average dis-

placement of the deflated dough was 231.2 ml., or 81.2 ml. more than the unfermented dough immediately after it was mixed.

Results and Discussion

For convenience in tracing these changes in volume, the averages of 34 sets of observations with a single formula and flour are recorded in the following table. Such values may be expected to vary with flour properties, dough formulas, and treatment or manipulation.

Volume of the Doughs at Several Stages of Manipulation

Treatment	*Volume ml/100 g flour*
As mixed	150.0
As punched and molded for proofing	193.0
As proofed for baking	535.0
After deflation in pressure flask	231.2
Reduction in volume by deflation	303.8

The average of the water manometer readings observed for 34 replicated tests was 96.9 mm. when the dough aliquot in the chamber or jar was prepared with 50 g. of flour. The system was then calibrated by placing a paraffin block having a displacement of 115.6 ml. (one-half of the 231.2 ml. indicated for a 100-g. dough in the above table) in the Mason jar, and then measuring the volume of gas that must be introduced to elevate the pressure to 96.9 mm. on the water manometer. This proved to be 4.85 ml. In other words, the deflation of the 50-g. flour dough from 267.5 ml. to 115.6 ml., or in the amount of 151.9 ml., involved the discharge of 156.75 ml. of gas from the dough as computed indirectly at atmospheric pressure. Inserting the resulting values into Boyle's law for pressure vs. gas volumes, and 737 mm. mercury as the average barometric pressure at the point of observation, the pressure of gas in the 151.9 ml. of space that it occupied in the dough was 760.53 mm. of mercury. This is 23.53 mm. of mercury above the barometric pressure of the dough environment, or the equivalent of 1.032 $\times$ atmospheric pressure at the point of observation.

It is evident from the data in the table that not all of the gas in the vesicles of the dough was discharged or "punched out" by the manipulation here involved in deflating the dough. No convenient mechanical or other device was available that could be expected to remove all such gas. One may assume that at least 231.2 ml. minus 150 ml. (the displacement of the dough as mixed—see table) or 81.2 ml. of gas (more as measured at atmospheric pressure, *viz.* 81.2 $\times$ 1.032 or 83.8 ml.) remained in the doughs on the average after the partial deflation effected by the moving plunger, and as computed with doughs made with 100 g. flour.

The question then arises as to whether this residual gas was actually under the same pressure as the (approximately) 80% that was actually removed or "punched out." To afford some estimate in this connection, a series of aliquots of the same dough were fermented to the same volume, and then subjected to progressively increasing degrees of deflation, approximating the discharge of 20, 40, 60, and 80% of the volume of gas in the proofed dough. Calculations based upon these observations disclosed that there was no major or consistent difference in the pressure of the increments of gas thus discharged. In other words, extrapolation of these data would lead to the assumption that the 20% of gas remaining in the dough after the most vigorous of these several deflation manipulations was under approximately the same pressure as all of the gas in the fully proofed dough. The final proof of this assumption admittedly is not yet available, however.

Obviously there are several variables that may merit study in this connection, such as the grade and gluten content of the flour, formula of the dough including various flour improvers, mechanical manipulations, length of the fermentation treatments, and the like. The correlation between data obtained from testing doughs by means of physical dough-testing devices and the pressure of gas held in the vesicles of the dough might prove to be significant. Merely varying the absorption or flour:water ratio might yield data of some importance. Possibly studies of this nature may be expanded to advantage, and the simple gas-pressure measuring device or chamber here described may prove useful in such connections.

Literature Cited

1. BAILEY, C. H., and JOHNSON, A. H. Carbon dioxide diffusion ratio of wheat flour doughs as a measure of fermentation period. Cereal Chem. 1: 293–304 (1924).

A NOTE ON JOJOBA BEAN MEAL – A POTENTIAL FEED[1]

FRANKLIN B. WELLS

The jojoba plant, *Simmondsia californica,* is an evergreen shrub which grows wild in Arizona, Lower California, and western Mexico. It prefers arid and often mountainous areas where it is eaten by browsing animals, and its fruit, a single bean in a thin, brown, three-valved capsule, is used as food by various rodents. Some commercial plantings of the shrub appear to have been made in recent years. The jojoba bean appears to be the only definitely known source of liquid wax. It contains 45% or more of an oil consisting almost entirely of a mixture of the esters of eicosenoic and docosenoic acids with eicosenol and docosenol. The oil and some of its uses have been described previously (4, 5).

Despite its rich oil content, the economics of the jojoba bean will depend to a large extent upon the value of the meal left after removal of the oil. The analyses reported in this paper were carried out to secure some indication of the potential value of the meal as a feed.

Jojoba beans, harvested in Arizona in 1942 containing 4.1% moisture and 1.25% ash, yielded 45.2% of oil on extraction of a ground sample with benzene for 100 hours. The resulting meal was analyzed for protein, carbohydrate, and ash by the usual A.O.A.C. methods (2), and the results, expressed both on the basis of the benzene-extracted meal and the original beans, were as follows:

	Oil Seed Meal %	*Whole Beans* %
Protein, (N × 6.25)	31.5	16.1
Carbohydrates		
Reducing sugars, as glucose	8.9	4.6
Nonreducing sugars, as sucrose	3.7	1.9
Other carbohydrates, as starch	16.0	8.2
Ash	3.08	1.57

A second extraction of the meal with benzene for 100 hours removed 0.6% more oil, and to obtain an oil-free meal, a final extraction was made with acetone for 100 hours.

Mineral analyses made on the melts obtained by igniting samples in a Parr sodium peroxide bomb are given in Table I.

The ash from the benzene-extracted and benzene acetone-extracted meal was hard and gritty and had a reddish-brown tint while that

[1] Manuscript received September 7, 1954. Contribution from the laboratories of Bloomfield College and Seminary, Bloomfield, N. J.

TABLE I
MINERAL ANALYSIS OF JOJOBA MEAL OBTAINED BY BENZENE AND BENZENE-ACETONE EXTRACTION

Constituent	Mineral Content, Dry Basis					
	Meal			Whole Bean Basis		
	Benzene-Extracted	Benzene Acetone-Extracted	Acetone Extract	Benzene Extracted	Benzene Acetone Extracted	Acetone Extract
	%	%		%	%	%
Sulfur	0.38	0.47	0.15	0.19	0.17	0.02
Calcium	0.56	0.78	0.00	0.29	0.29	0.00
Phosphorus	0.32	0.45	0.00	0.16	0.16	0.00
Ash	3.08	3.76	0.07	1.57	1.38	0.01

from the whole beans was a soft, light-gray powder. These differences in color and character and in the amount of ash indicate that a significant percentage of volatile inorganic matter was lost during the ashing of the whole beans. This is not entirely surprising in view of the work of Liebenthal (3) and of André and Carbouères (1).

The acetone extraction of the benzene-extracted meal yielded 13.7% (whole bean basis) of a gummy, aromatic substance resembling bee propolis in color, odor, and consistency; after 48 hours, this material became hard and brittle.

Since jojoba beans are eaten by rodents, it can be assumed that toxic materials are probably present only in minor proportions, if at all. Limited feeding trials with white rats gave satisfactory results. A pollen substitute consisting of a mixture of three parts ground jojoba bean solids with one part of dry nonfat milk solids gave equally good results as a standard pollen substitute consisting of three parts soybean flour and one part nonfat milk solids. Bees not fed either of these substitutes raised only about 65% as much brood during the same period.

The author and some of his co-workers ate various amounts (0.1 to 5.0 g.) of the benzene-extracted meal at four successive 24-hour intervals, followed by 10.0 g. of whole beans. At a later period, the author ate 25 g. of benzene-extracted meal and in no case were any ill effects observed. Both the meal and the beans possess a somewhat bitter flavor which has led to the colloquial name of "quinine nut."

These limited experiments indicate that jojoba meal has potential value as a feed, and further trials are warranted to determine its nutrient value and whether it is entirely nontoxic to domestic animals and humans. The bitter principle would probably have to be removed or modified to make the beans completely acceptable for human consumption.

Literature Cited

1. André, É., and Carbouères, Marie. Volatile sulfur in seeds of certain crucifers (forage cabbage). Compt. rend. **235**: 816–818 (1952).
2. Association of Official Agricultural Chemists. Official Methods of Analysis of the A.O.A.C. (7th ed.) (1950).
3. Liebenthal, F. Volatile silica affecting plant ash analyses. Science **114**: 636–637 (1951).
4. Wells, F. B. Process for making sulfurized jojoba oil. U.S. Patent 2,450,403 (Sept. 28, 1948).
5. Wells, F. B. Jojoba oil, a liquid wax, and some of its applications. J. Chem. Educ. **31**: 253–254 (1954).

BOOK REVIEWS

Brotgetreide und Brot (Neumann-Pelshenke), 5th edition. By P. F. Pelshenke, xii+622 pp. Paul Parey, Berlin and Hamburg, 1954. Price 76 marks.

This is the fifth edition in the series, the first edition of which was written by the late Dr. M. P. Neumann, Director of the Cereal Research Institute in Berlin, Germany, in 1914. Like the earlier editions, this volume covers a very wide range of subject matter. Beginning with a consideration of the origin and classification of the common cereals, it describes the structure and physical properties of the grains, cereal diseases and insect pests, respiration, storage and fermentation, grading standards, milling technics, baking equipment and processes, baking properties of flour and the occasion for variations in such properties including major chemical constituents and enzymes, and finally a section on bread as a nutrient material.

As is inevitable in covering such an extensive area of scientific and technological interest in the limited space of one volume, certain of the seven sections are somewhat brief and not exactly monographic in style and completeness. Also one regrets at times that the places and dates of publication of the journal articles to which references are made are not included in the citation.

It naturally follows that the major part of the discussion of cereal processing technics is based upon European practices. It is true, however, that frequent references are made to American practices, as, for example (p. 478), the use of chemical additives for maintaining the "fresh" or moist condition in the crumb of bread. And there are many other mentions of American and other non-German publications in the text discussions.

Of special interest to the reviewer is the excellent and concise presentation of the principles and mechanics of wheat conditioning or "tempering" (p. 255), which has been the subject of extensive study and development in Europe and England of late. Detailed chemical characteristics of flour mill streams, including their proteins and constituent amino acids, are also presented in the same section.

One is also favorably impressed by the logical completeness of Section V (p. 327 ff.), which is concerned with bread production. The mechanics of mixing and baking, physical properties of dough, biochemical changes in fermentation and the enzymes and organisms concerned, and many other details are covered in orderly fashion. This section should be of much service to students of this subject and particularly those who desire a knowledge of practices over a wide area.

The preparation of the manuscript of such a comprehensive text is truly a heroic task, and the author is to be commended upon the extent of the coverage, and the logical manner in which the information gleaned from consulting contemporary literature has been fitted together in the text. Dr. Pelshenke has certainly maintained the high standards of the earlier editions that were set by the late Dr. M. P. Neumann.

C. H. Bailey
Institute of Agriculture
University of Minnesota
St. Paul 1, Minnesota

Advances in Agronomy, Vol. VI, edited by A. G. Norman. xi + 383 pp. Academic Press, Inc., New York. 1954.

The sixth volume in this series continues the pattern of earlier volumes, with nine chapters, each devoted to progress in research in a segment of this broad field. The book has much material of value to the cereal chemist. The chapters are well organized and documented, with an extensive literature list. In some sections a few minor errors were noted.

The role of potassium in plant nutrition is reviewed by Kirk Lawton and R. L. Cook. Evidence is presented for the participation of this element in the synthesis of carbohydrates, proteins, organic acids, and oils as well as in cell division and photosynthesis. Deficiency symptoms, the potassium requirements of various crops, and problems in analyses for plant potassium are also included. O. J. Kelley deals with the requirement and availability of soil water with reference to field crops and fruits. A chapter on technological aspects of trace element usage by A. A. Nikitin covers the available sources, suitable salts, methods of incorporating in fertilizers, and problems in availability for plants.

Among new concepts of management of Corn Belt soils, S. W. Melsted lists the use of ample supplies of nitrogen fertilizers as one of the most important. Adequate nitrogen along with mulches, minimum cultivation, and chemical weed control may allow for improved soil management coupled with high yields. Over a wider area, C. P. Wilsie and R. H. Shaw consider the relation of the constituents of climate—moisture, temperature, light, and air movement—to the adaptation and evolution of crop plants. Progress in improvement of the Western range is presented by E. H. McIlvain and D. A. Savage. They describe developments in grazing management, reseeding, control of weeds, and supplemental feeding.

Two chapters are devoted to problems in the improvement of two important grain crops, sorghum and seed-flax. J. R. Quinby and J. H. Martin trace the development of combine types and earlier-maturing varieties and the wider use of sorghum in industry. Much is now known of the inheritance of seed color, sweetness of stalk, and the waxy, sugary, and yellow endosperm types. Preliminary field tests have already been made on hybrid sorghum produced through the use of male-steriles. The chapter on seed-flax is divided into sections, each handled by a competent authority. W. F. Geddes takes up the changes occurring in flaxseed in storage when the grain is high in moisture content. Included also are sections on flax rust, by H. H. Flor; other diseases, by J. J. Christensen; weeds, by R. S. Dunham; and breeding, by J. O. Culbertson.

The past decade has seen a substantial increase in international collaboration in agriculture. One chapter is devoted to a very readable account by J. G. Harrar of the program of technical assistance sponsored by the Rockefeller Foundation in Mexico and more recently in Colombia. The objectives and procedures are illustrated with the work on corn, wheat, and beans.

W. K. Smith
University of Wisconsin
Madison 6, Wisconsin

Das Getreide und seine Verarbeitung. Books 1 through 5. Versuchsanstalt für Getreideverwertung, Berlin, 1953.

These five books, which were published in 1953, comprise a bibliography of the various aspects of grain (excluded are brewing and distilling literature), organized in the following sections: Book 1 (green cover), planting and harvesting; Book 2 (blue cover), testing and evaluation; Book 3 (yellow cover), processing; Book 4 (red cover), physiology of nutrition; Book 5 (gray cover), economy and organization.

The bibliography is restricted to fundamental publications originating from universities or institutions with university standing. The years from 1900 to 1950 are covered, and only publications in German are considered.

Since most of the references listed are dissertations (Ph.D. theses or equivalent), their availability will be limited to those which have been published in book form. Some of these works are said to have appeared in university publications, but no direct reference to that is made. Inquiries for loans should be directed to public

libraries, but it is likely that most of the official university publications in which these references were published are in file in larger United States reference libraries.

Book 1 covers the following topics: History and geography of the grain; soil; fertilizers; water relationships and balances; seeds; varieties of seeds; breeding; cultivation techniques; the development of the grain plant; outside influences such as disease, climatic influences and needs, etc.; yield; harvest; machinery for raising wheat; author index.

HEINZ SORGER-DOMENIGG
University Farm
St. Paul, Minnesota

Monographs on Malt and Malt Products. The Identification of Threshed Barley. 18 pp. + table and plate. Prepared and presented by Muntona Limited, Bedford, and Edward Fison Ltd., Stowmarket, England, 1954. (Complimentary distribution.)

This monograph is the sixth of a series of publications on barley and malt. The physical features of the ear and grains of barley are described and the following features are chosen for identification of British varieties: 1) size, shape and color of the grain, 2) character of the rachilla, 3) character of the base of the grain, 4) serration and pigmentation of the lateral and mid-veins of the dorsal palea, and 5) character of the lodicules.

Sixteen two-row barleys and one six-row variety are described. A glossary is provided defining terms used in descriptions. The entire system of description is summarized in a single table where, through the use of symbols, the characteristics of each variety may be seen at a glance. Photographs of outstanding quality show grains of eleven varieties in dorsal, ventral, and lateral view, and illustrate clearly the external features of the grains of each variety.

R. F. PETERSON
Cereal Breeding Laboratory
Canada Department of Agriculture
Winnipeg, Manitoba

An Introduction to Industrial Mycology, by George Smith (4th ed.). 378 pp., illus. Edward Arnold, Ltd.: London, England.

The book has two introductory chapters on structure, classification, and nomenclature of fungi, several chapters that describe a total of 53 genera of fungi (seven Phycomycetes, seven Ascomycetes, 26 Fungi Imperfecti, and 13 Yeasts); a chapter devoted to each of the common genera Aspergillus and Penicillium; and a chapter each to physiology, control, and industrial uses of molds. The final chapter lists and briefly describes the journals devoted mostly to mycology and some of the principal monographs and texts in general mycology. Microscopy is presented in an appendix, followed by an adequate index. Literature pertinent to the subject matter is cited at the end of each chapter.

Most of the genera of fungi discussed are illustrated with photomicrographs, many of which are excellent, some of which illustrate little of the habits or characters of the fungi concerned. The descriptions of fungi are brief, clear, and informative, and although only 40 genera of fungi other than yeasts are included, this section of the book offers a good elementary introduction to the principal fungi of industrial significance. This reviewer would be inclined to question whether the chapters on physiology and on control of molds adequately introduce these large and complex fields to readers with no prior background.

The book is well printed on good paper and is of convenient size. It should be very useful to those with little or no background in mycology who wish to acquire elementary information on the lives, habits, and identification of fungi commonly encountered in industry.

CLYDE M. CHRISTENSEN
Department of Plant Pathology
University of Minnesota, Institute of Agriculture
St. Paul 1, Minnesota

Los Trigos de la Ceres Hispanica, by R. T. Molina and M. A. Pena. Instituto Nacional de Investigaciones Agronomicas: Madrid, Spain, 1952. 516 pp., illus.

This book is a compilation of varietal descriptions and classifications of wheats, published and unpublished, by Lagasca and Clemente, two Spanish botanists of the 19th century.

The first part of the book (48 pp.) is dedicated to the biographies of Lagasca and Clemente. It tells the story of the original manuscript of *The Spanish Cereals,* and gives a comparison of the species distinguished by Lagasca and Clemente with their present synonymy.

In the second part (392 pp.) in which the herbarium is described, there are color plates prepared under Lagasca and Clemente as well as new pictures of the most important samples from the original collection.

In the appendices are fragments of *Genera et Species Plantarum* (in Latin) and *On the Cast of Wheats* by Clemente. The latter appendix is the most important. It gives a good description of the wheat varieties that were grown in Spain including grain characters and color characters, and often indicates milling and baking qualities such as water requirements, sponginess, amount of ferment (yeast), and value of the flour for macaroni, spaghetti, cakes, and bread.

Besides their historical interest, the descriptions indicated above often can be utilized directly in many of the Latin-American countries where most of the wheats have not been improved since their introduction by the Spanish conquerors.

ALFREDO CAMPOS
The Rockefeller Foundation
Londres 45, Mexico 6 D.F., Mexico

Food Acceptance Testing Methodology. Edited by David R. Peryam, Francis J. Pilgrim, and Martin S. Peterson. 115 pp. Advisory Board on Quartermaster Research and Development, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois, 1954.

This report, one of a series entitled "Surveys of Progress on Military Subsistence Problems," is the first of Series III on Food Acceptance. It is comprised of papers and discussions given at a conference in Chicago, October 8–9, 1953. The book is divided into two parts, Part I dealing with "Panel Techniques" and Part II "Consumer Preference Techniques." The purpose of the conference as presented by Dr. Dean Foster, Chairman, was to define the objectives of food acceptance testing methods and to project possible solutions.

The papers in Part I review the single-stimulus, paired-stimulus, duo-trio, triangle, dilution, and flavor profile methods of testing and the variables affecting such tests. Their application to food products is well illustrated in the seven papers presented. Panel selection and training, some scoring methods, and the problem of sensory fatigue are also discussed in this section.

Four papers in Part II of the report stress the importance of laboratory testing to our military program. The discussions concerning the relation of laboratory panels to field testing is especially interesting. Two stimulating papers were those concerned with new psychophysical methods and reports on studies of psychophysics and the normality assumption. These new methods require some knowledge of statistics but provide another approach to the solution of problems in the field of food testing.

Both parts of the conference closed with general discussions of their respective topics, but it was felt that a more concrete summary of some of the problems that need investigation would be a worthy addition.

This publication will be of interest and value to those contemplating the establishment of a testing panel and an excellent review and challenge to those already engaged in this field of work. It is valuable because it clearly indicates the need for standardization of methodology, uniform methods of publication, and the importance and necessity of cooperation among those engaged in this work.

HELEN A. MOSER
Northern Utilization Research Branch
Peoria, Illinois

Soil, by G. V. Jacks, Director, Commonwealth Bureau of Soil Science, Rothamsted, England.

The preface of this interesting little book of 221 pages states the author's purpose—to give the farmer and the student of agriculture an insight into the new science of soil management.

The reader with only a limited knowledge of the basic sciences of chemistry, botany, etc., will obtain from this book a better understanding of various complex processes that are continually taking place in the soil. The practical agriculturalist seeking an explanation of processes involved in plant nutrition, utilization of plant nutrients when added to the soil in the form of fertilizers, and addition of soil amendments will have a better realization of various factors involved in plant growth.

The first chapter entitled "What is Soil?" is given considerable space for the size of the book. Many books of similar nature dismiss this subject with general statements that leave the reader in doubt as to what actually constitutes the soil; here, however, the subject is skillfully handled. This chapter has some excellent color reproductions of soil profiles, some of which have the characteristics of soils found in the United States.

The chapters on the chemistry of the soil and soil water are particularly helpful in explaining and clarifying some of the chemical processes and moisture relationships involved in plant growth. The chapter dealing with the biological aspects of the soil (including humus) emphasizes the importance of the micro-organisms and how they contribute to soil productivity.

The chapter on soil classification points out the difficulties of developing a scheme of classifying soils that is satisfactory from a genetic viewpoint as well as useful in a practical sense.

The book concludes with a chapter on the history of British soils, of particular interest to those whose knowledge of soils in foreign countries is somewhat limited.

P. R. McMiller
Department of Soil Science
University of Minnesota
St. Paul, Minnesota

ERRATUM

Cereal Chemistry, Vol. 31, No. 5

(September, 1954)

Page 371, Cuendet, Larson, Norris, and Geddes:

The photographs of the loaves of bread made from patent flour stored for 52 weeks at 3% and 14% moisture, respectively, are reversed; that is, the photograph for 3% moisture should be for 14% and that for 14% should be for 3%.

Cereal Chemistry

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for **Cereal Chemistry.**

Manuscripts for publication should be sent to the Editor in Chief. Advertising rates may be secured from and subscriptions placed with the Managing Editor, University Farm, St. Paul 1, Minnesota. Subscription rates, $11.00 per year. Foreign postage, 50 cents extra. Single copies, $2.50; foreign, $2.60. Back issues, $3.00.

SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see
Cereal Chem. **30:** 351–352 (1953).

The NEW Precision STERWIN FEEDER for dry powders

There's easy, accurate, trouble-free feeding of dry milling materials every time you set the feed-rate knob and throw the switch on the new Sterwin Feeder.

For the feed rate is controlled by a precise micrometer adjustment, assuring unprecedented accuracy and uniformity and requiring a minimum of operator's attention.

Precision-engineered specifically for the milling and feed industries, the Sterwin Feeder provides exact feeding of Vextram®, Oxylite®, Bromate mixtures, Malt, and other dry powders.

FEATURES OF THE STERWIN FEEDER

1. Transparent front door allows full view of operation.
2. Feed rates obtained by single adjustment knob.
3. Range: 4 oz. to 5 lbs. per hour at low speed, 3 lbs. to 60 lbs. at high speed. Special, easily changed gears for higher rates.
4. Floor space only 22" x 27" . . . height 27".
5. Transparent lid gives complete view of contents.

Low power requirements and simplicity of design make the Sterwin Feeder extremely economical to operate and maintain. And extreme uniformity of feeding reduces the overage ordinarily required to take care of feed-rate variations.

For complete information on the new Precision Sterwin Feeder . . . ask your Sterwin Technically Trained Representative or write:

IN CANADA:
Sterwin Chemicals of Canada, Ltd.
1019 Elliott Street W., Windsor, Ont.

Sterwin Chemicals Inc.
Subsidiary of Sterling Drug Inc.
Flour Service Division
1123-25 MERRIAM BLVD., KANSAS CITY, KANSAS
SPECIALISTS IN FLOUR MATURING, BLEACHING AND ENRICHMENT

The

American Association of Cereal Chemists

takes pleasure in announcing its

Fortieth Annual Meeting

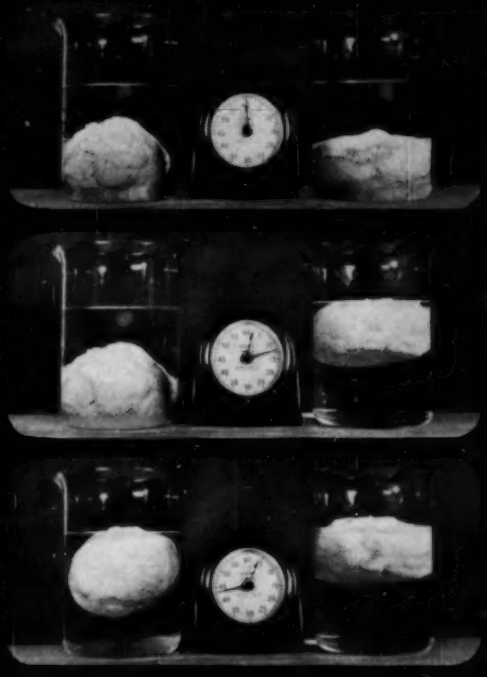
May fifteenth to nineteenth

at the

Chase Hotel, St. Louis, Missouri

Presidents Reception *Sunday Evening, May 15*

Proof PY-RAN* Leavens in the Oven!



Freshly mixed doughballs made with self-rising flour rest in liquid. Dough at left leavened with PY-RAN; another calcium phosphate in dough at right.

2 minutes, 12 seconds: PY-RAN dough remains at bottom. The other calcium phosphate has evolved so much CO_2 that doughball floats to top.

3 minutes, 44 seconds: PY-RAN dough finally begins rise, proof of slow controlled gas release during critical pre-oven period.

PY-RAN (anhydrous monocalcium phosphate) not only holds its leavening action for the oven, it gives longer shelf life to prepared mixes and self-rising flour. A special moisture-absorbent coating protects PY-RAN from reaction during storage. PY-RAN performs well with other leavening acids, gives superb texture, volume, crust and crumb color, reduces tunneling.

You will also like SAPP-40 for machine doughnut mixes, SAPP-28, a slow-action baking acid, and HT* Phosphate (MCP monohydrate). Ask the local Monsanto representative for the new book "Monsanto Phosphate Leavening Agents," or write: Monsanto Chemical Company, Inorganic Chemicals Division, 710 North Twelfth Boulevard, St. Louis 1, Missouri.

*Reg. U. S. Pat. Off.

MONSANTO

SERVING INDUSTRY . . . WHICH SERVES MANKIND

WORLD'S LARGEST PRODUCER OF ELEMENTAL PHOSPHORUS

EXTRA! Big News!

for everyone with a Pest Problem!

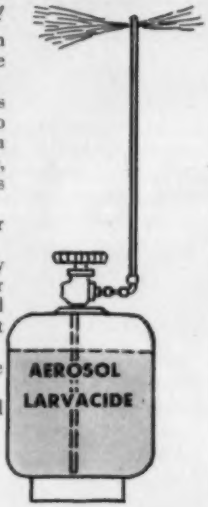
Now LARVACIDE in aerosol form is being applied in mill and storage space with instant gasification!

With AEROSOL Larvacide, it takes less time to fumigate, less time to aerate—and you get the wellknown LAVACIDE kill: insects, egglife, larvae. Rodents too, without carcass nuisance.

Rats are driven out of retreats, to die on the open floor where they're easily swept up.

AEROSOL Larvacide is released under pressure by simply opening a valve. The gas travels up to 50 feet in five or six seconds. Penetrates mill machinery, cracks and sacked goods. Gets into those remote retreats where pests might find refuge from fumigants less potent.

Look into AEROSOL Larvacide today. See how much more quickly and more easily it does the good job you want. *Write for Circular* "Your Guide to Effective and safer Mill Fumigation".



Liquid LARVACIDE is still available, in cylinders of 50, 100, 180 and 250 lbs. net, and those handy 1 lb. dispenser bottles, each in sealed can.

Larvacide Products, Inc.

117 Liberty Street, New York 6, N. Y.

STORAGE

OF CEREAL GRAINS AND THEIR PRODUCTS

J. A. Anderson
A. W. Alcock
editors

1954 • 6 x 9 • x + 515 pp. • 90 illustrations, 52 tables
1120 references

Price: $11.00

AMERICAN ASSOCIATION OF CEREAL CHEMISTS

University Farm — St. Paul 1, Minnesota

SHORTENINGS YOU CAN DEPEND UPON

PRIMEX B&C

An all-hydrogenated vegetable oil shortening of exceptional stability. Excellent for all deep frying purposes. Preferred by biscuit and cracker bakers, manufacturers of prepared biscuit, pie crust and doughnut flours, and makers of other food products where rancidity troubles are to be avoided.

SWEETEX

The "High-Ratio" shortening. Especially designed to permit bakers to produce "High-Ratio" cakes, icings, and sweet yeast goods with superior eating and keeping qualities.

NUTEX

An all-hydrogenated vegetable oil shortening combining the exceptional stability of Primex B&C and the "High-Ratio" properties of Sweetex. A top quality shortening manufactured for use in prepared cake, yeast raised doughnuts and sweet yeast dough, and icing mixes.

PRIMEX

The all-hydrogenated shortening "that sets the quality standard." A top grade shortening especially recommended for doughnut frying, for pies, cookies and bread, and for other shortening purposes.

PROCTER & GAMBLE

Branches and warehouses in principal cities

General Offices . . . . . CINCINNATI, OHIO

Cereal Chemists Find Flour Treated with HY-KURE* Gives Excellent Baking Results

"Lab" Tests Confirm Earlier Findings of Milling Chemists

Rigid laboratory tests by cereal chemists underscore the fact that flour matured and bleached by HY-KURE provides superior baking qualities.

Many bakers are now using flour treated by HY-KURE to produce bread with a finer, more silky texture, uniform grain and a clear white crumb color.

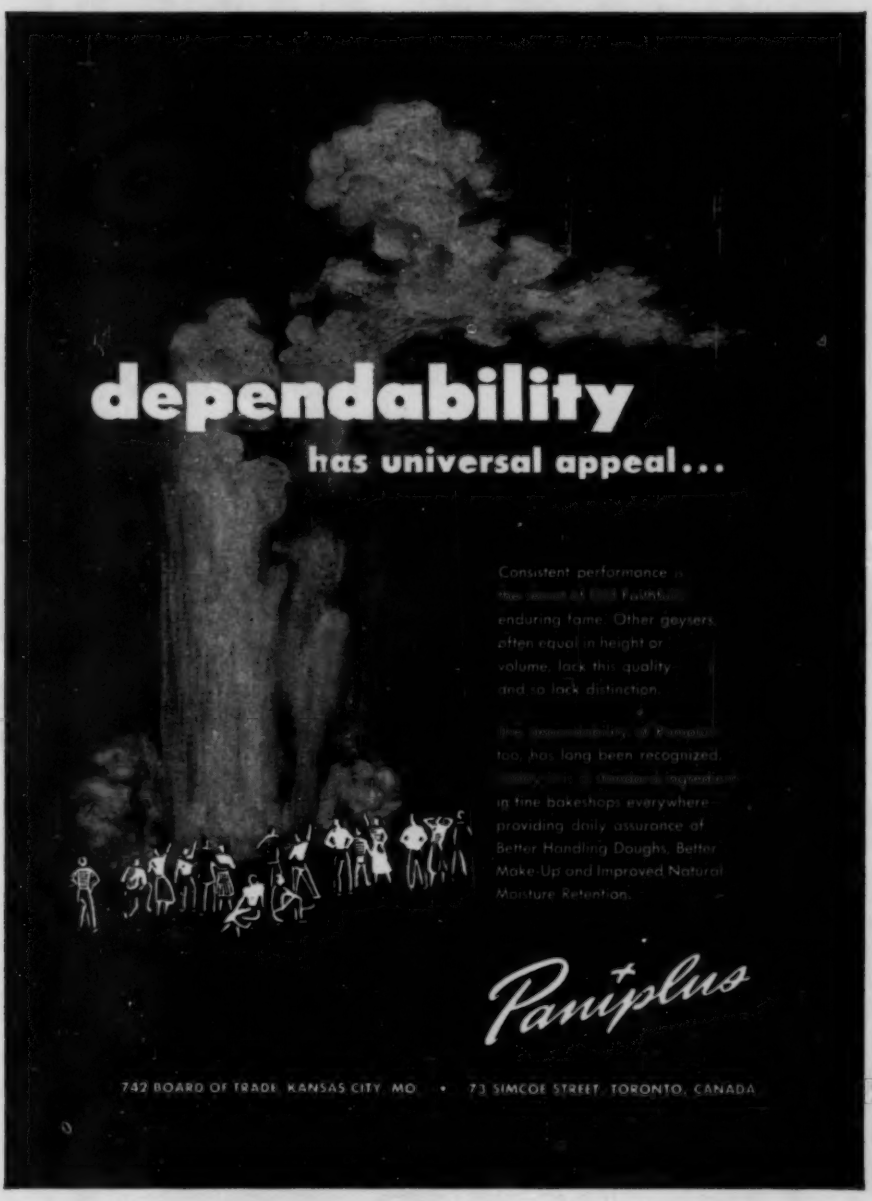
HY-KURE is the revolutionary, new way of supplying chlorine dioxide for the maturing and bleaching of flour. A major HY-KURE advantage is UNIFORMITY of flour treatment . . . bakers can secure more consistent baking results with flour processed by HY-KURE.

Why not learn how HY-KURE treated flour can help you produce better baked goods.

IN CANADA:
Sterwin Chemicals of Canada, Ltd.
1019 Elliott Street W., Windsor, Ont.

Sterwin Chemicals Inc.
Subsidiary of Sterling Drug Inc.
Flour Service Division
1123-25 MERRIAM BLVD., KANSAS CITY, KANSAS
SPECIALISTS IN FLOUR MATURING, BLEACHING AND ENRICHMENT

*TRADEMARK U.S. Pat. No. 2,683,651. Other Patents Pending.


dependability
has universal appeal...
Consistent performance
enduring fame. Other geysers
often equal in height or
volume, lack this quality
and so lack distinction.
too, has long been recognized
in fine bakeshops everywhere
providing daily assurance of
Better Handling Doughs, Better
Make-Up and Improved Natural
Moisture Retention.
Pamplus
742 BOARD OF TRADE, KANSAS CITY, MO. • 73 SIMCOE STREET, TORONTO, CANADA

when you use . . .

THE DYOX PROCESS® for flour maturing . . .
The fixed uniformity of DYOX treated flour is a definite advantage to the miller who strives to meet the baker's demand for a starting product of constant value—which works in the bake shops with the least trouble and manipulation by the operator. DYOX treated flour assures overall dependability, uniformity of product, and ease of operation.

"NOVADELOX"® for whiter, brighter flour . . .
"Novadelox" is designed to meet the desire of the consuming public for a bread that is truly white—and to enable millers to produce a uniform product of standard color.

"N-RICHMENT-A"® for uniform enriching . . .
"N-Richment-A" provides a readily available, simple and troublefree product for enriching flour—with the assurance that the standard procedure of adding "N-RICHMENT-A" will produce a dependable standardized flour.

THE N-A FLOUR SERVICE DIVISION with more than a quarter-century of experience . . .
The N-A Flour Service Division with its skilled laboratories and staff is always available to work with you or your consultants on all phases of maturing, bleaching and enriching. Why not phone your nearest N-A Representative today!

DYOX
for flour maturing
NOVADELOX
for a whiter, brighter flour
N-Richment-A
for uniform enrichment

NA-62

NOVADEL FLOUR SERVICE DIVISION
WALLACE & TIERNAN INCORPORATED
BELLEVILLE 9, NEW JERSEY
REPRESENTATIVES IN PRINCIPAL CITIES